SCIENTIFIC PROCEEDINGS.

Vol. XXVI. October, 1928. No. 1.

4106

Relation Between Colloid Osmotic Pressure and Concentration of Serum Proteins.

ELLA H. FISHBERG.

From the Chemical Laboratory of Beth Israel Hospital, New York City.

Since the initial communication of Starling,[1, 2] it has been established that the serum proteins exert an osmotic pressure. The same investigator showed later that the colloid osmotic pressure is approximately proportional to the concentration of protein in the serum. It has been found that as serum is diluted the colloid osmotic pressure falls more sharply than does the concentration of the serum proteins. Verney[3] believes that the explanation of this phenomenon is to be found in the comparatively large molecular volume occupied by the colloidal particles, the line of reasoning being analagous to that employed in deriving the pressure-volume relationship of highly compressed gases. He finds that, where p is the colloid osmotic pressure of the proteins, v the reciprocal of the protein concentration, b is a constant depending on the particular conditions, the formula $p(v—b) = k$ holds for dilutions up to 50% of that of normal serum. Thus he finds that the colloid osmotic pressure of the serum proteins varies with their dilution, within definite limits, in the manner that would be expected of an unionized colloidal solution in which the colloidal particles occupy an effective volume as large as 50% of the original.

It is thus known that the colloid osmotic pressure of the serum per gm. of protein becomes lower, the less the actual concentration of the protein in the serum. If we assume that the rate of change of the colloid osmotic pressure with respect to the concen-

---

[1] Starling, *J. Physiol.*, 1896, xix, 312.

[2] Starling, *J. Physiol.*, 1899, xxiv, 317.

[3] Verney, *J. Physiol.*, 1926, lvi, 319.

tration of the protein is proportional to the actual colloid osmotic pressure, we have the differential equation

$$\frac{dp}{dv} = ap$$

where p is the reciprocal of the colloid osmotic pressure and v is the reciprocal of the protein concentration. Separating the variables and integrating

$$\pm a.dv = \int \frac{dp}{p}$$

$$\log p = av$$

$$p = eav$$

where a is a constant, dependent on the initial concentration of protein in the normal serum. We have found this constant in human serum to have a value of 0.75. Applying this constant to the data obtained in our experiments (Table I), as well as that contained in the publications of Mayrs,[4] Krogh,[5] and Verney,[3] the agreement between the calculated values and those ascertained experimentally is striking.

TABLE I.

Relation of protein concentration to osmotic pressure.

Gm. protein in 10 cc. serum	Osmotic pressure Cm/100	v	p	p = eav av. calculated	p = eav av. measured
.721	.353	1.39	2.83	1.04	1.05
.639	.301	1.58	3.35	1.19	1.21
.577	.283	1.72	3.53	1.29	1.26
.505	.219	1.98	4.57	1.49	1.52
.432	.169	2.31	5.93	1.73	1.78
.361	.106	2.78	8.42	2.09	2.13
.289	.075	3.27	13.46	2.60	2.60
.216	.029	4.64	35.1	3.48	3.57

It is to be emphasized that the formula derived above can be utilized only for the calculation of the colloid osmotic pressure of successive dilutions of one and the same serum. It would seem that it cannot be used for the estimation of the colloid osmotic pressure of pathologic sera, in which, in addition to diminution of total protein concentration there is an abnormal relationship between the individual protein fractions.

Summary: The relationship between the concentration of protein in the serum and the osmotic pressure may be expressed by $p = e^{av}$

---

[4] Mayrs, *Quar. Med. J.*, 1925, xix, 285.

[5] Krogh, *Biochem. Z.*, 1927, clxxxviii, 255.

where p is the reciprocal of the osmotic pressure and v the reciprocal of the concentration of the proteins, that is, when the proteins decrease in arithmetical progression, the osmotic pressure decreases in geometric progression.

4107

Pupillary Reactions During Ether and Chloroform Anesthesia After Morphine.

HARRY GOLD AND MARCY L. SUSSMAN.

From the Department of Pharmacology, Cornell University Medical College.

In the course of anesthesia by ether and chloroform in the dog, the pupil dilates at 2 stages, in the period of excitement and in the period of deep narcosis. If there is violent excitement the intermediary constriction is not observed. It is commonly held that the primary dilatation is a reflex phenomenon (inhibition of the oculomotor or stimulation of the sympathetic or both). There is no clear statement, however, whether the dilatation of the pupil in the stage of deep narcosis is the result of asphyxia or the direct effect of the anesthetic. While studying the response of the vagus nerve to morphine during ether and chloroform anesthesia, it was observed that in deep ether narcosis, the pupils of the dog under the influence of morphine were widely dilated, while in deep chloroform narcosis under the same conditions, the pupils were constricted. These observations seemed of interest in their bearing upon the mechanism of the pupillary changes produced by ether and chloroform. Twelve experiments were then performed to check and extend these observations and the results are the subject of the present report.

Ether and chloroform were administered by inhalation with the open cone method. The pupils were measured under constant conditions of light for any given experiment. Both eyes were examined; the pupils were found equal in all except one instance. Electrocardiographic records of the heart rate were taken at frequent intervals, making it possible in this way to correlate simultaneous effects upon the vagus and oculomotor nerves. The condensed protocols of the experiments with 2 dogs serve to illustrate the general results. The size of the pupils is expressed in percentage of the diameter of the iris. The stage of anesthesia is designated as "light" when voluntary movements as well as corneal conjunctival reflexes are present; as "deep" when these have disappeared; as "partial recovery" when the eye reflexes have reappeared; as "recovery" when voluntary movements begin.

PROTOCOL I. Dog, male, weight 11.2 kg.

Time	Size of pupil	Heart rate	Stage of anesthesia	Remarks.
3:18	40	—	—	Chloroform started.
3:20	90	—	Light	Violent struggling.
3:23	90	—	Deep	Resp. nearly ceased; chloroform withdrawn.
3:30	40	—	Part. Rec.	Ether started.
3:32	90	—	Light	Moderate struggling.
3:34	90	—	Deep	Ether withdrawn.
3:37	70	—	Part. Rec.	10 mg. morphine sulphate per kg., muscle.
3:45	5	64	—	Panting at 200 per min.; winks; enophthalmus; chloroform started.
3:48	5	60	Deep	No struggling; resp. nearly ceased, Cheyne-Stokes; tongue cyanotic; enophthalmus; chloroform withdrawn.
3:58	5	—	Part. Rec.	—
4:05	5	70	Recovery	Ether started.
4:07	20	—	Light	Slight struggling.
4:10	90	116	Deep	Exophthalmus.
4:14	90	142	Deeper	Resp. shallow; exophthalmus; ether withdrawn.
4:20	40	—	Part. Rec.	—
4:37	5	120	Recovery	—

In the dog, morphine does not ordinarily produce the degree of constriction seen in man. It does, however, increase oculomotor tone, particularly in evidence when inhibitory influences are removed, as by sleep. Thus, in this case, there was marked pupillary constriction after morphine, probably because the animal was still sufficiently depressed by the previous anesthetic.

Protocol I shows that, while in the normal dog deep ether and chloroform anesthesia both dilated the pupils, after a large dose of morphine, the pupils were dilated in deep ether and constricted in deep chloroform anesthesia. After a relatively small dose of morphine, Protocol II, insufficient to abolish struggling when the anesthetic was inhaled, chloroform in the stage of excitement caused dilatation of the pupils, but constriction in the period of deep narcosis. Ether, on the other hand, caused constriction of the pupils in the early stage of administration (there was no struggling, the animal having been depressed by the morphine and the previous anesthesia) and caused marked dilatation of the pupils in the period of deep narcosis.

Morphine slowed the heart rate. After the large dose of morphine, the heart rate remained slow in deep chloroform anesthesia, but became rapid in deep ether anesthesia. After a small dose of morphine, the same general relationship obtained; the heart accelerated moderately in chloroform anesthesia and markedly in ether anesthesia.

PROTOCOL II. Dog, male, weight 17.72 kg.

Time	Size of pupil	Heart rate	Stage of anesthesia	Remarks
3:28	40	75	—	1 mg. morphine sulphate per kg., muscle.
4:05	30	60	—	Chloroform started.
4:06	90	—	Light	Violent struggling.
4:20	20	95	Deep	—
4:21	40	—	Lighter	Corneal reflex just returned.
4:25	10	100	Very deep	Resp. very shallow; tongue cyanotic; eyes closed; enophthalmus; chloroform withdrawn.
4:33	50	85	Part. rec.	Ether started.
4:43	20	—	Light	Breathing so shallow that very little ether inhaled; no struggling.
4:45	20	115	Deep	Corneal reflex just disappeared; resp. deeper and faster.
4:50	50	130	Deeper	—
4:55	75	140	As above	Resp. good, 48 per minute.
4:56	90	160	As above	Exophthalmus; eyes open; ether withdrawn.
5:01	90	—	Part. rec.	—
5:07	80	160	Recovery	—

These experiments present evidence in support of the following statements: (1) The cause of dilatation of the pupils in the excitement period of chloroform anesthesia is different from that in deep anesthesia, since under given conditions one of these effects can be abolished and the other retained. (2) The dilatation of the pupils in the period of deep anesthesia is not the result of asphyxia but the direct effect of the anesthetic (probably direct depression of the oculomotor).

The parallel behavior of the cardio-inhibitor and the oculomotor is interesting. Morphine increases the tone of both nerves. After a large dose, chloroform, which did not depress the oculomotor, did not depress the cardio-inhibitor; while ether, which depressed the one, also depressed the other. After a small dose of morphine, however, the cardio-inhibitor was apparently more readily depressed than the oculomotor.

It seems probable that the difference shown between ether and chloroform in these experiments depends upon the fact recorded by Straub,[1] that morphine potentiates the toxicity of chloroform on the respiratory center. That the pupils dilate and the heart accelerates during deep chloroform anesthesia without morphine, but fail to do so after morphine, may be explained by the fact that in the latter case the animal dies of respiratory paralysis before a concentration of chloroform in the body is reached sufficient to depress the cardio-inhibitor and oculomotor nerves.

---

[1] Straub, W., *Munch. med. Wochenschrft.*, 1913, lx, 1823.

4108

Bacteriology of Blood and Joints in Chronic Infectious Arthritis.

RUSSELL L. CECIL, EDITH E. NICHOLLS AND WENDELL J. STAINSBY.

From the Cornell Clinic and the Second (Cornell) Medical Division of Bellevue Hospital.

During the past 2 years, we have been making a bacteriological study of patients suffering from chronic deforming arthritis, giving special attention to cultures of the blood and joints.

The technique employed for blood cultures is as follows: 20 cc. of blood are taken aseptically from the arm with a Luer syringe, placed in 2 sterile culture tubes, allowed to clot, and put in the ice-box overnight. In the morning the tubes are centrifuged, and the serum drawn off with a sterile pipette. Care is taken to remove all the serum. The clot is then broken up in the original tube with a sterile glass tube, diameter ¼ inch, drawn up in the same tube, and transferred to a 3 oz. bottle containing 50 cc. broth (beef-heart infusion broth, 0.5% saline, 1% peptone, pH 7.6). The two bottles are then put in the incubator at 37° and left there unopened for 5 days.

At the end of this time, 2 tubes, each containing 8 cc. of a 1.5% beef-infusion-broth-agar, are melted, partially cooled, and 0.5 cc. of whole rabbit's blood added to each tube. Each tube is then seeded with a loopful of broth culture and the contents poured into a petri dish. The plates are allowed to incubate for 24 to 48 hours. Pour-plate cultures are repeated at 3 day intervals for 4 weeks unless the colonies appear sooner. All contaminated cultures are discarded.

In the 56 cases studied of chronic infectious arthritis with swelling, 47 presented one or more characteristic fusiform fingers, while the remaining 9 had one or more swollen joints in various parts of the body. Of the 56 cases, 38 or 68% showed an atypical *Streptococcus* in the circulating blood. In the 14 cases studied of chronic infectious arthritis without swelling, 7, or 50%, were positive. 43 controls without exception yielded negative results. These controls included 16 cases of chronic degenerative arthritis, 6 rheumatic heart disease, 1 gonococcal arthritis, 1 gout, 13 patients having various other infections without joint symptoms, and 5 normal healthy individuals.

Cultures from joints and lymph glands were made in the same manner except that rabbit's blood instead of human blood was added to the bottles of broth. Cultures were made from involved joints in

6 cases. In 2 of these the material was curettings from the head of the femur; in another 2 it was synovial membrane of the knee joint; and in the remaining 2 cases, the synovial membrane from fusiform fingers was cultured. In the 2 cases cultured from bone-curettings, an atypical *Streptococcus viridans* was recovered identical morphologically and culturally with the organisms recovered from the blood. The remaining joint cultures yielded no organisms.

Two enlarged lymph nodes which were taken from patients suffering from this disease yielded sterile cultures.

The most striking characteristic of these streptococci is the very slow growth of the first generation in broth. The average time of appearance of the streptococci in sub-culture was 15.7 days, but ranges from 4 to 30 days. In blood broth cultures, they cause slight turbidity of the broth, and a heavy granular sediment, with a small amount of hemolysis of the blood. The supernatant broth takes on a greenish tinge. On blood-agar plates, the colonies appear as small greyish dots, surrounded by a narrow, partially hemolyzed zone of greenish tinge. The hemolysis is much smaller in amount than that observed in a typical strain of *Streptococcus hemolyticus*.

All strains are insoluble in bile. None ferment inulin and mannit. All ferment dextrose, salicin, and lactose.

At the present time agglutination studies on the various organisms isolated are being carried out. A diffuse growth of the streptococci is obtained by growth in potato broth. Immune sera are obtained by injecting rabbits at 4 day intervals with 20 cc. of a 24 hour broth culture heated for one hour at 56°. This work has not been completed to date, but immune sera from 6 rabbits, immunized against 6 strains, strongly agglutinate all other strains with a very few exceptions. The evidence so far obtained tends strongly to confirm the theory that chronic deforming arthritis is an infectious disease caused in the majority of cases by a biologically specific strain of streptococcus.

4109

Transmission of Respiratory Anaphylaxis (Asthma) From Mother to Offspring.*

BRET RATNER AND HELEN LEE GRUEHL.

From the Departments of Immunology and Pediatrics, University and Bellevue Hospital Medical College, New York University.

We have recently demonstrated[1] that when guinea pigs are subjected to an organic dust-laden atmosphere, after a certain incubation period, they will become highly sensitized to the substance which they have inhaled. Prior to our work, it was thought that nasal sensitization could be brought about only through the insufflation of solutions. It was our privilege to prove that this sensitization could be induced through the inhalation of a dry substance (horse dander). We ventured the opinion that this particular demonstration proved that respiratory anaphylaxis in the guinea pig and bronchial asthma in the human being should be regarded as identical conditions.

In a further series of experiments[2] we have shown that a guinea pig sensitized prior to or during pregnancy will sensitize her offspring *in utero,* and that this mechanism is dependent entirely upon the permeability of the placenta.

Thus we have shown 2 ways in which sensitization may be brought about; first by the absorption of inhaled dry substances through the upper respiratory tract; and second by the transmission of sensitizing substances from the circulation of a pregnant mother to the circulation of the fetus.

In the present work, we have taken guinea pigs before and during pregnancy, and produced respiratory anaphylaxis (asthma) in them. We studied 26 guinea pig families in all, and in 20 of these we were able to produce respiratory anaphylaxis in the mother. In 18 of these families this respiratory anaphylaxis was transmitted to the offspring, as was proven both by exposing them to the dust, to which they reacted without any previous form of sensitization, and by injecting them with an extract made from horse dander, from which they either died in typical anaphylactic shock or developed

---

* This work is being carried on under "The Crane Research Fund for the Study of Allergic Diseases in Children."

[1] Ratner, B., Jackson, H. C., and Gruehl, H. L., *Am. J. Dis. Child.*, 1927, xxxiv, 23.

[2] Ratner, B., Jackson, H. C., and Gruehl, H. L., *J. Immunol.*, 1927, xiv, 249, 267, 275, 291, 303.

profound anaphylactic symptoms. In 6 of the families, we were unable to produce respiratory anaphylaxis in the mother and in 2 of the families, respiratory anaphylaxis was induced in the mother, but was not transmitted to the offspring.

The establishment of respiratory anaphylaxis has been adequately controlled by innumerable experiments, for in no instance has an animal been utilized who showed the slightest evidence of dyspnea during its sensitization period, and it is only after the incubation period has elapsed that respiratory anaphylaxis is manifested. The new-born animals were adequately controlled by the six negative groups, indicating that even animals as young as one day old show no dyspnea when placed in a cage unless they are sensitive.

That a dry substance entering the respiratory tract may go into solution in the maternal circulation, traverse the placental barrier, and enter the fetal circulation is demonstrated by the above experiments, and may offer another explanation[3] for the transmission of allergy or anaphylaxis from a mother to her baby. This transmission of hypersensitiveness from a mother to her offspring we must regard as strictly congenital.

4110

Changes in the Peripheral Circulation Accompanying "Tobacco Angina."

ELAINE P. RALLI AND B. S. OPPENHEIMER.

From the Cardiographic Laboratory of Mount Sinai Hospital, New York.

It is now fairly well established that coronary artery disease, occlusion, and consequent myocardial infarction constitute one of the pathological conditions associated with the syndrome, *angina pectoris.* There is, however, great diversity of opinion as to the mechanism of this symptom-complex; the probability is that typical *angina pectoris* is due to a single mechanism. There are several well recognized exciting causes of angina; such as physical effort, emotional excitement, exposure to cold or to fresh air, overeating, and in some patients tobacco smoking. We had observed a very few patients, beyond middle age, predisposed to *angina pectoris,* in whom the attacks could be provoked very promptly by smoking a cigarette. The attacks of pain induced by smoking were relieved by administering a nitroglycerin tablet dry under the tongue, but were not relieved by a tablet similar in appearance but not containing any nitroglycerin. The relief of pain was, therefore, not due to a psychic factor; no attempt was made to substitute any form of smoke other than that of tobacco. These few patients, therefore, offered an opportunity to study the peripheral circulation before, during, and after an attack of *angina pectoris*. This was done by making observations of any change in limb volume by means of a plethysmograph on the left arm, and simultaneously of the systolic and diastolic blood pressures in the other arm by the ausculatory method.

---

[3] Ratner, B., *Am. J. Dis. Child.*, 1928, xxxvi, 277.

A simple air plethysmograph was used. This was made of glass and was approximately 13 inches long; the diameter at the open end was 5¼ inches, and the jar tapered down so that the diameter at the closed end was 1½ inches. The open end was covered with thick rubber membrane into which a small opening was cut, so that the patient could insert his hand and forearm without any undue pressure. A glass tube led from a rubber stopper at the top of the plethysmograph, and this was connected by rubber tubing to a glass tube containing water. In this way after an even baseline was established, changes in volume in the plethysmograph could be readily observed, but for the sake of simplicity no attempt was made to record these volumetric changes by means of a bellows recorder and smoked drum, or other apparatus.

Observations were made on 6 patients, all giving a history of precordial pain; also on 2 normal individuals, one male and one female, as controls. One typical example will be given here.

Male patient, baker, 45 years of age, formerly a heavy smoker, whose resting blood pressure was elevated, averaging 165/100-110 mm., would experience a typical attack of *angina pectoris* following 4 or 5 deep inhalations of a cigarette. (Deep breathing alone did not excite an attack.) Each attack consisted of precordial pain, radiating into the left arm, with *angor animi,* ashy color, cold perspiration. This pain was associated with a marked rise in both the diastolic and the systolic blood pressure, and by evidences of peripheral *vasoconstriction* as registered by the plethysmograph. The peripheral constriction *preceded* the onset of pain by 15 to 45 seconds. There was a marked rise of systolic and diastolic blood pressures (of about 40 mm. of mercury) which followed the peripheral vaso-constriction but just preceded the onset of pain. Following the administration of 1/100—1/50 grain of nitroglycerin tablet dry under the tongue, the plethysmograph would give evidence of peripheral dilatation which preceded the cessation of pain.

The interval of time elapsing between the peripheral dilatation and the cessation of pain depended somewhat on the severity of the pain. Three separate sets of observations were made on this patient, and in each case the results were the same.

In three of the 6 patients there was definite evidence that first peripheral vaso-constriction, and later a rise in arterial tension preceded the onset of pain. In 2 other cases, there was evidence only of an elevation of systemic blood pressure, but not definitely of peripheral vaso-constriction. The sixth patient refused to smoke, but exercise with a foot ergograph resulted in a rise in arterial tension with each attack of pain, but there was only slight evidence of peripheral vaso-constriction.

There are, therefore, instances of patients predisposed to *angina pectoris,* in whom cigarette smoking induces a peripheral vaso-constriction, which precedes the onset of precordial pain. At the same time an elevation of blood pressure was observed, so that it is reasonable to suppose that there was a general vaso-constriction, in which the coronary arteries may have taken part.

4111

Studies on Inhibition of Insulin Activity.

SAMUEL KARELITZ, PHILIP COHEN AND SIDNEY D. LEADER.
(Introduced by Bela Schick.)

From the Pediatric Department and Laboratories, Mount Sinai Hospital.

In the fall of 1925 at the suggestion of Dr. Bela Schick we investigated a diabetic child with refractory periods to insulin. The study of this case led us to investigate the inhibitory effect of blood on insulin activity.

Our experiments have all been done with rabbits. Unless otherwise stated, all work was done as follows. One physiological or 3 clinical units of insulin were mixed with the substance investigated and incubated at 37° C. from 1 to 2 hours, then injected subcutaneously into rabbits, weighing 2 kilos, starved during the preceding 24 hours. Hourly blood sugar determination and clinical observations were made.

The results summarized were as follows:

2-4 cc. of human plasma caused hardly any inhibition of insulin action.
5 cc. of human plasma caused moderate inhibition of insulin action.

10 cc. of human plasma caused marked inhibition of insulin action.
15 cc. of human plasma caused complete inhibition of insulin action.
2 cc. of centrifuged unwashed blood cells caused mild inhibition.
5 cc. of centrifuged unwashed blood cells caused marked inhibition.
5 cc. of human plasma from diabetics caused almost complete inhibition.
2 cc. cells of case of myeloid leukemia caused complete inhibition.
2 cc. pus of case of empyema caused complete inhibition.

When more than one physiological unit of insulin was used, 10 cc. of plasma caused only slight—questionable inhibition. Controls of saline and insulin treated as was the plasma, etc., showed no inhibition. Likewise a few experiments with agar agar, egg albumen, and 5 cc. of horse serum (purified), treated as was the human plasma or cells, showed no inhibitory effect.

pH controls revealed that the plasma inhibited at pH 7.4 to pH 8.2 but did not inhibit at pH 6. Normal saline solution could inhibit insulin if brought to pH 9 or over by addition of NaOH. There was no difference observed whether Lilly, Squibb or Stearns insulin was used.

Inhibitions due to plasma was observed even when incubation was short (15-30 minutes), also when kept at room temperature, 20°-25° C., rather than at 37°.

Desiring to study inhibition of insulin action in infection, we followed the reaction to insulin after rabbits were injected with typhoid or staphylococcus vaccine, believing that after vaccination the reaction of the blood is like that of infection. About 50% of the animals showed moderate decreased insulin effect 3 hours after vaccine injection. This inhibitory action lasted several days in some animals. Our studies with blood from patients with high fever, serum sickness, high leucocytosis, acidosis or lipemia are too few to permit of any definite conclusions.

Our results suggest that there is something in human blood plasma which is more abundant in blood cells, more in diabetic blood than in normal human blood, and still more in leukemic cells and pus which inhibits or destroys the action of insulin. It is probably more abundant in blood of patients with fever, leucocytosis, suppurative processes, or serum sickness, than in normal state. The extent of inhibition is variable for individuals, but quantitatively proportional to the amount of blood used.

We are of the impression that the inhibitory substance may be a proteolytic enzyme, because the inhibition is greater with blood cells than with plasma, greater with leukemic cells and pus than with normal blood cells, and because it seems to be stronger during fever or infection or after vaccine injection. Even more significant, in that it may be enzyme action, is the experiment in which the plasma or pus heated at 57° for 1 to 2 hours no longer inhibited insulin action. Similar experiments with blood cells were not as convincing as was plasma or pus.

4112

Standardization of Liver Extract.

STANLEY D. BEARD, GUY W. CLARK AND MAX J. MOSES.

From the Lederle Antitoxin Laboratories, Pearl River, N. Y.

When rabbits are injected intranarrowly with *B. welchii* toxins according to the Torrey-Kahn[1] technique, they rapidly become anemic and exhibit many of the characteristic blood changes described in reports of pernicious anemia in man.

There is a rapid decrease in the number of red cells (for 5 rabbits: 31, 32, 38, 38, 41%) and in hemoglobin content (for 5 rabbits: 29, 30, 30, 31, 37%), the lowest values being reached between the fifth and tenth days. Following this, there is a compensatory period of 10 to 15 days during which time the experimental animals show definite increase in the number of red cells and hemoglobin. Beginning with about the thirtieth day after injection of the toxin, the experimental animals show a progressive decrease in the number of red cells and in the hemoglobin content.

If an aqueous solution of liver extract is given by stomach tube to these anemic rabbits while they are at the lowest point in red count and hemoglobin (about the fifteenth day), there is a definite increase in each. This is, however, the period in which the animals are making a strong effort to compensate for the injury to the hematopoietic system and one does not obtain such striking results as when liver extract is given in the stage of progressive decline (35 days, or later).

There can be no doubt as to the beneficial effects of liver extract in this type of experimental anemia. Our results with some 20 animals indicated a rough proportionality between the amount of liver extract given and the increase in the number of red cells and in

---

[1] A paper by John C. Torrey and Morton C. Kahn, "Progressive Effects of a Single Intra-tibial Injection of *B. welchii* Toxins," read at the joint meeting of the American Association of Pathologists and Bacteriologists and the American Association of Immunology, Washington, D. C., May 1st, 1928. We are greatly indebted to Drs. Torrey and Kahn for many suggestions.

TABLE I.

Comparison of Red Count and Hemoglobin of Anemic Rabbits with and without Liver Extract (expressed in % of original normal values).

Rabbit No.	Red cells	Hemoglobin (Dare)	
	%	%	
108	90	97	Received 68 gm. of liver extract in 42 days
110	87	97	Received 78 gm. of liver extract in 43 days
111	88	98	Received 78 gm. of liver extract in 43 days
113	67	80	Control—no liver
120	73	76	Control—no liver

the hemoglobin content. This at once suggests the use of such experiments as a means of standardizing commercial liver extracts. Further work is in progress.

4113

Secondary Rays from Lipiodol and Bismuth Subnitrate Paste on Staphylococcus aureus and Bacillus coli communis.

MARGARET E. FRIES AND MAURICE LENZ. (Introduced by Bela Schick.)

From the Pediatric Department and the Laboratories of Mt. Sinai Hospital.

Attempts have been made to inhibit the growth of microorganisms by using secondary rays. These secondary rays are emitted from metals when they are exposed to x-ray. It has been shown that the higher the atomic weight of a metal, the greater is the bactericidal power of the secondary ray.[1, 2, 3] Many substances have been used with varying clinical success by different investigators.[4-10] Holthusen[11] has pointed out that there are many difficulties in the

---

[1] Halberstaedter, L., and Meyer, P. S., *Fortschr. d. Rontgenstr.*, 1922, xxiv, 489.

[2] Milani, E., and Donati, C., *Radiologia medica*, 1921, viii, 417.

[3] Ghilarducci, F., *Xla riunione della societa italiana per il progresso delle scienze*, Trieste, sett., 1921.

[4] Beck, Emil, *Med. J.*, 1908, xiii, 402.

[5] Christen, T., *Stahlentherapie*, 1912, i, 51.

[6] Ridlon and Blanchard, *Am. J. Orth. Surg.*, 1908, vi, 13.

[7] Lynah, H. L., *J. Am. Med. Assn.*, 1921, lxxvii, 1548.

[8] Abbe, *Am. J. Roent.*, 1922, lx, 152.

[9] Friedrich, W., und Bender, M., *Strahlenther*, 1920, i, 11.

[10] Gudzent, F., *Strahlenther*, 1920, xi, 277.

[11] Holthusen, H., *Ergebn. d. med. Strahlenforsch*, 1925, i, 383.

clinical application of secondary rays. The amount of the substance acting as radiators must be relatively large and still non-toxic. The action of secondary rays is limited, since they are so easily absorbed.

Experiments were conducted with Lipiodol and Beck's paste as the radiators. These substances are non-toxic, contain a metal of high atomic weight (33% Bismuth subnitrate in Beck's paste and 40% metallic iodine in Lipiodol) and have been previously used for their therapeutic value in certain clinical conditions.[4, 13-17] *Staphylococcus aureus* and *Bacillus coli communis* were used as the test bacteria.

The bacteria were grown on agar slants for 18 to 24 hours and emulsions were made containing approximately 3,000,000 and 1,200,000,000 organisms per cc. Of the first emulsion 0.2 cc. was pipetted on to a dry sterile agar plate which had been incubated for 48 hours, of the second emulsion 0.1 cc. was used. With a glass spatula the emulsion was spread evenly over the surface of the plate. These plates were dried in the incubator for 10 minutes before exposure to the x-ray. The inoculated agar plate was turned up side down and the substance acting as radiator was placed 1 mm. below the surface of the agar growth. The x-rays, therefore, first entered the bottom of the glass dish, next penetrated the agar, then the surface culture and finally struck the substance which was being investigated.[11, 12, 18] The secondary rays from the latter were reflected on to the surface of the growth and either caused inhibition of the growth or none at all. Ten erythema doses of x-ray were given in 30 minutes with the following factors: Transformer machine, 200 K. V., 4 M. A., 30 cm. focal distance. No filter. The plates were then incubated at 37° and the results read 20 hours later.

It was found that secondary rays from bismuth subnitrate completely inhibited the growth of *Staphylococcus aureus* and *Bacillus coli communis* under these conditions. Secondary rays from Beck's paste and Lipiodol, on the other hand gave practically negative results. In those cases in which the results were doubtful, it was usually due to some error in technique. Of 18 experiments with Lipiodol, 15 did not show any inhibiting influence and in 3 it was

---

[12] Liechti, A., *Klin. woch.*, 1924, 825.

[13] Sicard and Forestier, *Bull. et Mem. d. Hoh. de Paris,* 1922, xlvi, 463.

[14] Archibald, *Can. Med. Assn., J.*, 1925, xv, 1000.

[15] Ballon, D., *Can. Med. Assn. J.*, 1925, xv, 995.

[16] Pritchard, S., Whyte, B., Gordon, J., *J. Am. Med. Assn.*, 1928, lxxxvi, 1119.

[17] Fiessinger, N., et Lemaire, A., *Presse Med.*, 1926, xxxiv, 209.

[18] Holthusen, H. Schubsck, und Sielman, H., *Strahlenther,* 1927, xxiv, 557.

negligible. In 9 experiments, the Lipiodol was in contact with the bacterial growth and even in these cases, the inhibitory effect of the secondary rays was only slight. Control experiments with secondary rays from iodine crystals or iodine tincture were impossible, as the vapor itself from iodine and 10% iodine tincture, at a distance of 1 mm. killed the bacteria. Of 8 experiments with bismuth subnitrate paste (Beck's paste—33%), 7 were negative and 1 doubtful. Of 14 control experiments with bismuth subnitrate powder, complete inhibition of growth was obtained in 13, and moderate inhibition in one.

Conclusions: Since the iodine vapor itself and the secondary rays from bismuth subnitrate kill bacteria, and since the secondary rays from the oily preparations of both these substances have no inhibitory effect, it would appear that the concentration of the metals in these preparations is not sufficient under the conditions of these experiments or that the fat interferes with the effect of the secondary rays.

The practical significance of these experiments is that Lipiodol and Beck's paste cannot be used therapeutically for the production of bactericidal secondary rays; for as has been shown the secondary rays emitted from these substances, under the conditions described, do not inhibit the growth of bacteria.

4114

Action of Ephedrin on Intestine and Bronchi.

J. T. HALSEY.

From the Department of Pharmacology, Tulane University.

Chen[1] and Kreitmair[2] believe that the pharmacological action of ephedrin in small doses is identical with that of adrenalin, that is, a stimulation of the sympathetic myoneural junction (commonly spoken of as nerve endings), while that of larger doses is due to direct stimulation of smooth muscle comparable to that produced by barium. Among other effects described by these investigators, are those of inhibition of the intestinal movements and relaxation of spasm of bronchial muscle. The former of these effects has been questioned by Nagel,[3] who failed to obtain any inhibition of the gut but solely a pressor effect.

---

[1] Chen, K. K., and Schmidt, C. F., *J. Pharm. Exp. Ther.*, 1924, xxiv, 339.

[2] Kreitmair, H., *Arch. f. Exp. Path. u. Pharmakol.*, 1927, cxx, 189.

Like Nagel, we have not (in over 100 experiments) obtained any evidence of a depressant action of ephedrin on the isolated rabbit gut although we have meticulously followed the methods and employed the dosage of Chen and Kreitmair. Other publications[4, 5] have called in question the view that ephedrin produces certain of its effects by sympathicometic action. The question is not of merely theoretical interest for if ephedrin does not stimulate the sympathetic nerve endings in the gut, it is highly unlikely that it stimulates similar inhibitory nerve endings in the bronchi. As such assumed pharmacological action is the basis of the use of ephedrin as a substitute for adrenalin in the treatment of asthma, a repetition of Chen and Kreitmair's investigations appeared desirable. In a limited number of experiments conducted in this laboratory, a broncho-dilating effect of ephedrin has been observed only exceptionally. Perhaps the divergent reports of the efficacy of ephedrin in bronchial asthma may have their explanation in the fact that ephedrin relaxes bronchial spasm only under certain conditions.

4115

Post Operative Blood Chemical Studies.

KNUTE REUTERSKIOLD AND EDMUND ANDREWS.

From the Surgical Department, University of Illinois, and Surgical Service, St. Luke's Hospital, Chicago.

In a series of 50 experiments on dogs operated under ether anesthesia, various blood chemical observations were made at short intervals. The findings were as follows: 1. There is a fall in the CO_2 (van Slyke) generally not more than 10 points. 2. The blood sugar rises slightly at first followed by a moderate fall—15-20 mg. 3. There is a rise in the concentration of the blood as measured by the refractometric index. This is slight and inconstant. 4. Leucocyte, temperature, pulse and blood pressure observations show moderate changes, but in none of our cases were these marked enough to be significant *per se*.

---

[3] Nagel, A., *Ibid.*, 1925, cx, 129.

[4] De Eds, F., and Butt, E. M., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 800.

[5] Gradinesco, A., *C. R. d. Soc. Biol.*, 1927, xcvi, 1027.

These observations were made at intervals of 15-30 minutes for 24 hours after the operation and as can be seen they totally fail to explain the profound state of collapse in which any postoperative case lies for about 24 hours.

The positive findings in our series are as follows: 1. A marked tissue thirst is indicated by the findings in the Aldrich intradermal salt test. During the period of postoperative lethargy the wheal is absorbed in approximately half the time that it took in the controls. This tissue dehydration is in striking contrast to the comparatively slight changes in the water content of the blood. 2. The most striking feature found was the enormous changes in the calcium and potassium in the blood. The Ca-K ratio was enormously lowered in every case, in some actually falling below 1. Return to normal had usually taken place by 24 to 36 hours. 3. Parallel with these mineral salt changes there occurs a decisive change in the permeability of the skin as measured by the Blister method. The blistering time is enormously prolonged, in severe cases to twice that noted just before operation.

Summary: Enormous changes in the Ca-K ratio in the blood are found after surgical operations under ether anesthesia. This is accompanied by an increased permeability of the tissues as well as a tissue dehydration.

4116

Uretero-ureteral Anastomosis.

WARNER S. BUMP AND S. M. CROWE. (Introduced by E. Andrews.)

From the John Jay Borland Fellowship, Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago.

The accidental division or crushing of ureters during difficult gynecological operations has occurred occasionally, even with the most experienced surgeons. Suture of the divided ureter when the level of division has been too high to allow implantation in the bladder has resulted in a low percentage of permanently functioning ureters. This failure has resulted most often from progressive cicatricial stenosis due to leakage of urine. In this study the method used by McArthur[1] for the plastic repair of a traumatically ruptured ureter is applied to end to end suture of the ureter in dogs and in one woman. Through a slit in the side of the proximal portion of the ureter a catheter is inserted distally toward the bladder and over it the ureteral ends are joined by 2 fine catgut sutures. Through the same slit in the ureter a snugly fitting rubber catheter is inserted toward the kidney to divert the urine from the site of suture for 8 days or more until healing occurs. The free ends of both catheters are brought outside the body in the lumbar region. The ureters of 6 dogs examined at intervals of 19 to 270 days after such an operation healed without narrowing or appreciable dilation of the lumens, with a minimum scar, without changes in the renal pelves, and without evidence of any considerable damage to the kidneys. Five months after a similar operation in a woman the ureter is slightly dilated but is not constricted at any point. The renal pelvis and calyces are not appreciably altered.

---

[1] McArthur, *Surg. Gynecol. Obst.*, 1925, xli, 719.

4117

Experimental Immunization of Horses with Herpes Virus.

EARL B. McKINLEY.*

From the Biological Division of the Bureau of Science, Manila.

It is well known that various strains of herpes virus produce encephalitis in rabbits. Some investigators believe that the herpes virus is the etiologic agent in epidemic encephalitis in human beings. At present there is little evidence to support this view. The fact remains, however, that the herpes virus has been recovered from the brain tissue of several cases of epidemic encephalitis and the question naturally arises as to whether the herpes virus is related to the disease process in such cases or whether it is merely present by accident as a contaminating agent. A critical discussion of the question is contained in another publication.[1]

In a previous communication[2] we have shown that rabbits may be hyperimmunized with herpes virus attenuated by fresh rabbit serum and that the serums from such rabbits are capable of producing passive immunity in healthy rabbits when injected intravenously. Attempts to immunize sheep were unsuccessful even when fresh virus was administered subcutaneously.

---

* Member of Field Staff, International Health Division, Rockefeller Foundation.

[1] McKinley, E. B., *Phil. J. Science*, in press.

[2] McKinley, E. B., and Holden, M., *Arch. Path. and Lab. Med.*, 1927, iv, 155.

The possibility of immunizing horses against the herpes virus occurred to us as a possible means of throwing some light upon the true etiology of epidemic encephalitis. If the serums from such horses proved virucidal for the herpes virus *in vitro* and were capable of producing passive immunity in rabbits when injected intravenously, the possibility of treating cases of epidemic encephalitis would present itself. While such a therapeutic test for etiology would not be conclusive evidence it might throw some light on the question, particularly if the cases so treated were properly controlled with other cases receiving normal horse serum.

In December, 1927, immunization of 2 horses with herpes virus "H. F." and herpes strain "Beckley" was begun. Both horses received injections of fresh saline emulsions of herpetic rabbit brains from rabbits which had just died of encephalitis. Injections were given at 3 day intervals subcutaneously. The first 4 injections consisted of 2 cc. each; the 5th of 3 cc.; the 6th of 4 cc.; followed by 6 injections of 5 cc. each. After the initial 12 inoculations each horse received 3 injections of an entire herpes infected rabbit brain emulsified in 50 cc. of salt solution given at 5 day intervals. In each case the emulsion was tested on rabbits and proved infectious. There were no reactions noted in either horse following the inoculations. Two weeks after each horse received the final inoculation both horses were bled and the 2 serums were brought to the laboratory for testing.

Neither the serum from the horse receiving the "H. F." strain nor the serum from the horse which had received the "Beckley" strain possessed any virucidal properties for the 2 strains of herpes virus employed in the experiment. Furthermore the anti-"H. F." herpes serum did not produce the slightest degree of immunity in normal rabbits when injected intravenously. Rabbits receiving 3 injections of 5 cc. each of this serum intravenously and subsequently tested subdurally with the virus died invariably within 4 to 6 days with typical symptoms of encephalitis. Furthermore the virus was recovered from these test animals when emulsions of the brains were inoculated into other healthy rabbits.

The anti-Beckley herpes serum was found unsuitable for immunizing rabbits. As small a quantity as 1 cc. of this serum when injected intravenously into rabbits produced convulsions and death of the rabbits within a few minutes. Subsequently it was discovered that a few drops of this horse serum when added to 5 cc. of citrated rabbit blood produced complete hemolysis within a few minutes. This phenomenon is not unknown and has been met with by other workers.

It appears from these experiments that suitable immune serums cannot be produced in horses against the herpes virus. Due to a paucity of cases of epidemic encephalitis in the Philippines we have been unable to test either of these serums in patients having this disease. From these experimental data it would appear that there is little scientific basis upon which to base such clinical tests.

4118

Experimental Herpetic Encephalitis Produced by Feeding Herpes Virus to Guinea Pigs.

EARL B. McKINLEY.*

From the Biological Division of the Bureau of Science, Manila.

Herpetic encephalitis may be produced in rabbits by inoculating such animals either subdurally, intravenously, subcutaneously, or intraperitoneally. The possibility of inducing herpetic encephalitis in guinea pigs by feeding occurred to us. While there is little evidence to support the view that epidemic encephalitis in man is caused by the herpes virus, any evidence regarding the portals of entry of this virus into the animal body is important. Nothing is known concerning the portal of entry of the true virus of epidemic encephalitis and epidemiological control of the disease is based largely upon speculation. It is known that paralysis follows the inoculation of the virus of poliomyelitis into the stomach and intestines of monkeys and it seemed probable to us that infection might be produced in this way with the herpes virus.

Four guinea pigs were fed by catheter 3 cc. of a freshly prepared emulsion of herpetic (Beckley) rabbit brain. One guinea pig received 4 cc. while a 6th received 5 cc. Of the 6 guinea pigs so treated 3 died. The animals which had received 4 cc. and 5 cc. of herpes emulsion respectively died on the 8th day following feeding. One of the guinea pigs which had received 3 cc. of the herpes emulsion by mouth died on the 10th day. The other 3 guinea pigs remained unaffected.

The brains of the 3 guinea pigs which died were removed and emulsions were prepared. Rabbits inoculated with these emulsions subdurally developed typical symptoms of herpetic encephalitis and died of the disease. Cultures prepared from the brains of the guinea pigs and inoculated rabbits were without growth. The herpes virus passed through guinea pigs by way of the gastro-intestinal tract and then passed back to rabbits appears to be somewhat attenuated at first but quickly regains its virulence. For example the virus recovered from the brain of the guinea pig which had received 5 cc. of herpes emulsion by mouth required 9 days to produce a fatal encephalitis in the first rabbit tested. When the virus from this rabbit was passed to 2 healthy rabbits subdurally both animals died with typical symptoms on the 5th day following the inoculation. Cultures prepared with the brains from both of these animals remained sterile.

---

* Member of Field Staff, International Health Division, Rockefeller Foundation.

It appears from these experiments that the herpes virus passes from the intestinal tract of guinea pigs to the central nervous system under these experimental conditions and that the virus may be recovered from the brains of guinea pigs following death of the animals and that the emulsions of such brains when inoculated into rabbits subdurally produces a typical herpetic encephalitis.

4119

Experimental Rinderpest in Goats.

EARL B. McKINLEY.*

From the Biological Division of the Bureau of Science, Manila.

The study of the virus of rinderpest has been rendered difficult because of the lack of a suitable experimental animal. The virus of rinderpest is pathogenic for cattle, carabaos, hogs, boars, sheep, goats and camels. With the exception of goats and sheep the other susceptible animals are either too expensive to use for experimental purposes or are unobtainable. That goats are susceptible to the virus of rinderpest has been shown by Kolle and Turner[1] and by Topacio.[2] In the Philippines where rinderpest has a high incidence among cattle it is possible to obtain goats in large numbers and they are even less expensive than rabbits. As a rule the mortality in goats from this disease is very low. This animal, however, manifests very definite symptoms of the disease. These symptoms consist of a gradual rise in temperature reaching a maximum of 40.2 degrees C. to 40.8 degrees C. on the 5th or 6th day. About this time the animal develops a diarrhoea which persists for some time while the temperature gradually returns to normal if the animal is to recover. Meanwhile the animal eats little or refuses to eat at all and there may be considerable loss in weight. Occasionally under experimental conditions a goat succumbs to the infection. The rule, however, is for the animal to recover.

---

* Member of Field Staff, International Health Division, Rockefeller Foundation.

[1] Kolle and Turner, *Z. f. Hyg.*, 1898, xxvii, 45.

[2] Topacio, *Phil. Agr. Rev.*, 1922, xv, 229; *Ibid.*, 1926, xix.

In searching for an experimental animal in which to study the virus of rinderpest we have attempted to infect chickens, rabbits, guinea pigs and monkeys. All of these animals were found to be immune to the virus. Regardless of the quantity of virus administered and of the route of inoculation (subdural, intravenous, subcutaneous and intraperitoneal routes were tried) there were no reactions whatsoever. As much as 100 cc. of virus infected carabao blood has been given to monkeys without producing any manifestations of the disease. Rabbits have received as much as 20 cc. of virus intravenously without any indication of infection.

We have found that goats are susceptible to the virus of rinderpest in very high dilutions. According to Boynton[3] the smallest quantity of rinderpest virus which will infect cattle is 1/2960 cc. In goats we have found that 2 cc. of a 1/10,000 dilution of virus infected carabao blood is sufficient to provoke a typical infection. On the other hand the same dilution of virus induced delayed symptoms of the disease in Davao cattle which are considered to be more resistant to the disease than are carabaos.

Goats were infected with rinderpest and passed through the disease, returning to good health. Subsequently these goats were infected again in order to ascertain if they were immune. We have been interested in determining if the serums from goats immune to rinderpest are virucidal for the virus of rinderpest *in vitro* as is known to be the case for immune serums in several other filterable virus infections. Several goats which had successfully passed through an attack of experimentally induced rinderpest and had subsequently been found to be immune were bled. The rinderpest virus used in these experiments consisted of infected carabao blood which was taken from the infected animal about the 5th day at the peak of the temperature curve. For each cubic centimeter of rinderpest infected carabao citrated blood in small test tubes were added 4 cc. of rinderpest immune serum from goats. Controls were prepared with normal goat serum, both fresh and inactivated, and also with fresh and inactivated rabbit serum. After allowing the mixture to stand for 24 hours at room temperature, following frequent shaking, healthy goats were inoculated subcutaneously with 2 cc. of each mixture. In every case the inoculated animals developed typical symptoms of rinderpest. In other experiments the mixtures were incubated at 37.5° C. for 6 hours before testing but there proved to be no virucidal activity of the immune goat serums.

---

[3] Boynton, *Phil. J. Sci.*, 1928, xxxvi, 1.

Rabbits were injected intravenously with 20 cc. of rinderpest infected carabao blood and 24 hours later were bled from the heart, the blood being immediately injected subcutaneously into susceptible goats. In no case did rinderpest develop.

It appears from these experiments that goats are excellent animals for the experimental study of rinderpest; that goats which have recovered from rinderpest are immune to subsequent attacks of the disease but that the serums from such goats possess no virucidal properties for the virus of rinderpest *in vitro* when virus and immune serums are mixed in a ratio of one to four. Further it appears that while fresh rabbit serum is nonvirucidal *in vitro* for the rinderpest virus this virus is either quickly destroyed or entirely eliminated from the blood stream of healthy rabbits following intravenous injections of large quantities of rinderpest infected carabao blood known to provoke the disease in goats in dilutions of 1/10,000. Experiments of this nature are facilitated by studying the disease experimentally in this animal.

4120

Chloroform-Treated Tissue-Vaccine in Experimental Herpetic Encephalitis.

EARL B. McKINLEY.*

From the Biological Division of the Bureau of Science, Manila.

During the past year Kelser[1] has prepared a chloroform-treated tissue-vaccine for rinderpest which is highly efficacious in protecting cattle and carabaos against this disease. Kakisaki, Nakanishi and Nakamura[2] in Japan have also prepared a tissue-vaccine for this disease by treating the infected tissues with toluol, ether, etc. The vaccine prepared by the Japanese workers is difficult to administer because of its consistency and is also absorbed very slowly. In the Philippines the Kelser vaccine has now been adopted as the standard method of protection against rinderpest and its efficiency approaches 100%. In more recent work Kelser[3] has prepared a chloroform-treated tissue-vaccine for rabies.

---

* Member of Field Staff, International Health Division, Rockefeller Foundation.

[1] Kelser, *J. Am. Vet. Med. Assn.*, 1927, xxiv, 97; *The Military Surgeon*, July, 1927; Paper F. E. A. T. M., Calcutta, December, 1927; *Phil. J. Sci.*, 1928, xxxvi, 4.

Briefly the chloroform-treated tissue vaccine for rinderpest as prepared by Kelser consists of finely ground tissues (lymph glands, tonsils, spleen, liver) from animals killed in the acute stages of rinderpest. This tissue is diluted with equal parts of physiological saline and for each 100 cc. of tissue emulsion 0.75 cc. of chloroform is added. Such a vaccine can be used a few hours after preparation. Kelser prepared a tissue vaccine for rabies by the same method except that in this case he employed the infected brains of rabbits dying of rabies. Vaccinated rabbits were found to be immune to subdural inoculation of rabies virus after 2 and 3 subcutaneous injections of the chloroform-treated brain emulsion.

In the field of the filterable viruses we can learn much by comparing the properties of one virus with those of other viruses. We have attempted to immunize rabbits with chloroform-treated brain tissue vaccine prepared with herpes virus. Brains from rabbits dying of experimental herpetic encephalitis (Beckley strain of herpes was employed) were emulsified in physiological salt solution according to the method of Kelser and treated with chloroform in the quantities recommended by this author. After 24 hours the vaccine was administered subcutaneously to rabbits and in all 4 injections of 3, 3, 4 and 4 cc. each were given. After 10 days following the last inoculation the rabbits were tested subdurally with 0.2 cc. of a fresh emulsion of herpes infected rabbit brain. Contrary to the results reported by Kelser for rinderpest and for rabies none of our animals were protected and all developed typical herpetic encephalitis and died on the 5th or 6th day. Rabbits tested with the chloroform-treated brain vaccine by the subdural route remained normal.

Kelser believes that in rinderpest the chloroform destroys the virus, since injections of the vaccine into susceptible animals produces no symptoms. We are inclined to the view that the virus is not destroyed but rather attenuated or mitigated by the action of this reagent. Immunity to filterable viruses in general has only been produced by living viruses and if killed virus in rinderpest produces immunity it stands alone as an example of this type. Kelser believes that some by-product in the tissue, perhaps of the nature of an aggressin, is responsible for the immunity produced. It seems more likely that a mitigated virus is responsible. We are unable to explain our results with herpes virus in view of Kelser's results with rabies virus but it is possible that herpes virus is more susceptible to the action of chloroform than are either the rinderpest virus or the rabies virus. In other words the herpes virus is probably destroyed by the action of this reagent and being so destroyed it is not capable of producing immunity. On the other hand since it has been suggested that some tissue product is responsible for the immunity produced by chloroform-treated tissue vaccines it appears that similar products are not present in the brains of rabbits dying of herpetic encephalitis.

---

[2] Kakisaki, Nakanishi and Nakamura, *J. Jap. Soc. Vet. Med.*, 1927, vi, 107.

[3] Kelser, (1928) in press. Personal communication.

4121

Leptospira Icterohaemorrhagiae in Wild Rats of the Philippine Islands.

EARL B. McKINLEY.*

From the Biological Division of the Bureau of Science, Manila.

In so far as we have been able to learn epidemics of so-called infectious jaundice have never occurred in the Philippines. Indeed the writer has been unable to find any record in the various hospitals in Manila or in the Naval Hospital at Canacao of a single case of infectious jaundice associated with the presence of the *Leptospira icterohaemorrhagiae*. This is a curious observation in view of the widespread prevalence of this disease in Japan and Formosa and the extensive shipping communications which exist between these countries and the Philippines.

The wild rat is known to harbor this parasite in other parts of the world. In Japan nearly 50% of wild rats are infected and the disease is quite prevalent particularly from September to November each year. In the United States Jobling and Eggstein[1] found 10% of the wild rats caught in Nashville infested with this organism. Walch and Walch-Sorgdrager[2] have found 33% of rats in

---

* Member of Field Staff, International Health Division, Rockefeller Foundation.

[1] Jobling and Eggstein, *J. Am. Med. Assn.*, 1917, lxix, 1787.

[2] Walch and Walch-Sorgdrager, *Am. J. Hyg.*, 1927, vii, 393.

Baltimore harboring the organism, while Langworthy and Moore[3] report 40% to 60% for Albany. Brill[4] has reported 20% of field mice positive in flooded districts in Germany. Noguchi has reported the presence of this parasite in rats caught in New York City and from time to time there have been clinical reports of the disease emanating from that city. Only recently Cushing[5] has reported 2 cases of infectious jaundice in New York City from which cultures from the urine yielded the organism in both cases and from the cerebrospinal fluid in one case.

While it now appears that the *Leptospira icterohaemorrhagiae* and the so-called *Leptospira icteroides* may be identical organisms and that the latter may be etiologically unrelated to yellow fever,[6, 7] it still remains that from the clinical standpoint infectious jaundice is an important disease, not only on its own account but because of its similarity to yellow fever. It becomes then quite important to know the distribution of this parasite.

Nearly a year ago we began a systematic examination of the wild rats caught in and around Manila in order to determine the presence or absence of this parasite. Since most of the shipping to the Philippine Islands comes to Manila we believe that rats caught in this vicinity are representative of those elsewhere in the islands in so far as this question is concerned. During this period we have examined 250 rats which were caught alive and brought to the laboratory for examination.

Our method of examination has consisted of (1) bleeding the rats from the heart (usually the blood from 6 rats was pooled) and injecting guinea pigs intraperitoneally with from 5 to 8 cc. of the pooled citrated blood; (2) the urines from each rat if possible were examined under dark field for spirochetes; several samples of the pooled citrated blood were examined by dark field; (3) half of each kidney was emulsified in physiological saline and examined under the dark field following which guinea pigs were inoculated intraperitoneally with the emulsion; (4) and finally the other half of each kidney was fixed in formalin and sections were prepared and stained according to Levaditi's method for examination.

In rat series 192 to 197 the pooled blood contained spirochetes and guinea pigs receiving 5 cc. of blood intraperitoneally from this series of rats developed typical jaundice and cultures of *Lepto-*

---

[3] Langworthy and Moore, *J. Infect. Dis.*, 1927, xli, 70.

[4] Brill, *Münchener med. Woch.*, 1927, lxxiv, 1537.

[5] Cushing, *J. Am. Med. Assn.*, 1927, lxxxix, 1041.

[6] Sellards, *Am. J. Trop. Med.*, 1927, vii, 71.

[7] Stokes, Bauer and Hudson, *J. Am. Med. Assn.*, 1928, xc, 253.

spira icterohaemorrhagiae were obtained from the kidneys of the dead guinea pigs. Healthy guinea pigs inoculated with kidney emulsion of these animals developed jaundice. The cultures from the original guinea pigs unfortunately became contaminated and were lost. We were never able to use these cultures for experimental infections and did not attempt to isolate others since we were interested solely in determining the presence of the parasite. Subsequent examination of over 50 rats has never resulted in another positive result. None of the kidney sections have shown spirochetes. From the morphological, pathological and cultural characteristics of these spirochetes we feel certain that they are *Leptospira icterohemorrhagiae*.

Schüffner and Kuenen[8] have shown that leptospira are found to be more prevalent in old rats than in young rats. Fully one third of the rats examined by us were young rats. It is doubtful if more than one rat of the 6 composing series 192 to 197 was infected in view of the apparent rareness of the infection in our whole series. This, of course, cannot be determined.

The paucity of the *Leptospira icterohemorrhagiae* in the wild rats of the Philippines is in harmony with the clinical records regarding infectious jaundice. One may estimate that probably not more than 1% of the Philippine rats are infected with this parasite and perhaps this figure is high. Certainly infectious jaundice is not a clinical problem in the Philippine Islands and in view of this study there appears to be an adequate explanation for this.

4122

A Method for Experimental Production of Chronic Abscess of Lung.*

LOUIS G. HERRMANN AND ELLIOTT C. CUTLER.
(Introduced by Peyton Rous.)

From the Laboratory of Surgical Research of the Western Reserve University School of Medicine and the Lakeside Hospital, Cleveland, Ohio.

In the attempt to produce in an experimental animal an abscess of the lung that is identical with the postoperative pulmonary abscess in man, we considered the factor of chronicity to be of major importance. In the work of Cutler, Holman, Schlueter, Holloway and Weidlein[1, 2, 3, 4, 6] the abscesses produced by the embolic method were all of the acute type and healed spontaneously within 3 weeks after the embolus containing the bacteria had been set free into the general circulation. Van Allen, Fox and Colle[5] showed the importance of cough in the prolongation of the suppurative processes in the lung. Because spirochetes, fusiform bacilli and other anaerobic organisms of the mouth are so frequently found in the chronic abscesses of the lung in man it was thought advisable to study the behavior of these anaerobic organisms when implanted in the pulmonary tissues *by means of an arterial embolus.* In previous experiments it was determined that when these organisms were introduced *by way of the bronchi* chronic pulmonary abscess was not produced.

---

[8] Schüffner and Kuenen, *Nederl. Tijdschr. v. Geneesk.*, 1923, ii, 2018.

* Presented at the Fifteenth Reunion of the Peter Bent Brigham Hospital, Boston, April 27, 1928.

Method: Series 1. 24 dogs. Material taken from the gum margins of a patient with *pyorrhea alveolaris* was placed within a segment of vein and the ends of the vein tied with silk suture. The experimental embolus thus produced was forced into the venous circulation through a glass cannula by means of normal saline solution from a syringe. Fresh preparations of the material used in these septic emboli were examined with a dark-field microscope and a large number of spirochetes and various types of fusiform bacilli and innumerable other motile and non-motile organisms were found. Cultures of this material revealed the presence of both aerobic and anaerobic micro-organisms. In each of the 24 animals an abscess developed at the site of the lodgment of the experimental embolus. The progress of these experimental pulmonary lesions was followed by frequent roentgenologic examinations. Ten animals of this series are still living and show roentgenologic evidence of a localized pulmonary lesion. The other 14 animals died or were sacrificed at various intervals of from 5 to 15 weeks. For example, Dog 39 was sacrificed at the end of 101 days and was found to have a localized chronic abscess of the lung which, as far as gross and microscopic examinations are concerned, is identical with the chronic pulmonary abscess that is found in man. The wall of this abscess was about 4 mm. thick and the cavity of the abscess was

---

[1] Cutler, E. C., and Schlueter, S. A., *Ann. Surg.*, 1926, lxxxiv, 256.

[2] Holman, E., Weidlein, I. F., and Schlueter, S. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 266.

[3] Schlueter, S. A., and Weidlein, I. F., *Arch. Surg.*, 1927, xiv, 457.

[4] Holloway, J. W., Schlueter, S. A., and Cutler, E. C., *Ann. Surg.*, 1927, lxxxvi, 165.

[6] Weidlein, I. F., and Herrmann, L. G., in press.

[5] Van Allen, C. M., Fox, R. A., and Colle, H. G., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 485.

lined with a dark gray necrotic membrane. The contents of the abscess had a very foul odor. Cultures of the material from the cavity of the abscess of this animal were made on 12 different kinds of culture media and grown under 3 different forms of anaerobiosis besides the usual aerobic cultures. We were unable to grow aerobic organisms from this material but we were able to isolate 5 different anaerobic organisms which were obtained in pure culture. Each of the 3 types of anaerobiosis yielded the same group of organisms. These pure cultures of anaerobic organisms have been used in the same manner as the original material from the gum margins of the patient with *pyorrhea alveolaris* and some of these pure cultures of anaerobes have given rise to chronic abscesses of the lung that were similar to the lesion described for Dog 39. A further report on the end results of these experiments will be published later. In the rest of the 14 animals from which pathologic specimens were obtained at autopsy definite localized lesions were found which were similar to the chronic lesion described for Dog 39.

Evidence is therefore submitted that the chronicity of the experimental abscess of the lung in dogs is dependent upon the presence of a certain group of anaerobic organisms commonly found at the gum margins of patients with *pyorrhoea alveolaris*.

4123

Relation of Dietary to Effects of Relatively Large Amounts of X-Rays on the Organism.

LOUIS H. JORSTAD AND EDWIN C. ERNST.

From the Research Laboratories and the Department of Radiology, the Barnard Free Skin and Cancer Hospital, St. Louis, Missouri.

In a study of the effects of x-rays on animals fed normal dietaries, diets varying in the content of vitamins A and B and diets varying in relative amounts of the basic constituents, protein, fat and carbohydrates, certain findings have been obtained.

As reported formerly,[1] 10 MAM (13 "e" units) of x-rays given twice a week to a full grown or growing rat, prolongs and increases growth on a deficient vitamin B ration. 25 MAM (34 "e" units) increase growth and lengthen life on a ration deficient in either vitamin A or vitamin B. On 50 MAM (68 "e" units) the increase in normal growth and length of life is more marked in the series fed a dietary deficient in vitamin A than in one fed a dietary deficient in vitamin B. When larger doses than the above are given, such as 75 MAM (102 "e" units) or 100 MAM (136 "e" units) the life of the animal is shortened on either of these deficient rations. On a balanced laboratory ration life is shortened in each case, the degree of shortening being in direct ratio to the amount of x-rays given. On a stock ration consisting of dog biscuits, lettuce, carrots, meat and corn, the average length of life of a 90 gm. rat with the latter dosage is 90 to 120 days.

---

[1] Burrows, M. T., Jorstad, L. H., and Ernst, E. C., *J. Am. Med. Assn.*, 1926, lxxxvii, 86.

As discussed in the communication cited above, these observations indicate that x-rays act to cause a protoplasmic breakdown within the cell. The apparent stimulation with the smaller dose is due to a predominance of the protoplasmic disintegration from which the energy of life is derived. When larger doses of x-rays are given, the greater splitting off occurs between the protoplasm and the ergusia, or vitamin A. With the smaller dose the splitting off occurs between the water soluble elements of the cell and the intercellular substances. In that a high vitamin A dietary does not wholly protect against these larger doses other structural elements of the cell must be destroyed also. This is illustrated by the observation that the usual balanced vitamin dietary protects for a shorter period of time than the well rounded stock ration.[2]

Our present studies indicate that if in addition to the stock ration a certain amount of vitamin A is added, greater increase in growth rate and further prolongation of life is noted. With the addition of cream to the dog biscuits in the usual laboratory ration, the growth rate reaches a certain maximum at 50 days, and then remains at this level until a period of 180 days. At this time a slight loss in weight occurs, but the animals are still living 215 days after the beginning of the experiment. This can be contrasted to the weight curve of a rat on a diet to which has not been added the cream. In this case the maximum increase in weight is reached at 40 days and from that time there is a gradual decline until death at 90 to 120 days. On a similar ration in which 3% cod liver oil is substituted for the cream, there is a slow but gradual increase in the weight curve after the 30 days period following the beginning of the x-ray dosage and this gradual increase in the weight curve has continued throughout the course of the experiment, 215 days. These animals appear normal to date. Former studies have shown that an increase in the vitamin A fraction alone does not protect these animals against the destructive action of the x-ray. These latter studies seem to indicate that the destructive action of the ray is prevented in direct proportion to the amount of fat soluble vitamin added to a well balanced diet. With our present source of vitamin A we are not able to introduce enough in the system so as to protect absolutely against higher dosages, because with the giving of 125 MAM (170 "e" units) twice a week the protective action of 3% cod liver oil in the ration is not marked. At present we are studying the effects of these dietaries to which is added a vitamin A concentrate. The question arises as to whether the body is able to utilize or metabolize these high concentrates.

---

[2] **Burrows, M. T., Jorstad, L. H., and Ernst, E. C., *Radiology*, March, 1928.**

It was found in our studies of x-ray burns that a well marked burn will protect an animal against a vitamin A deficiency for a considerable period of time.[3] These rats did not develop a xerophthalmia until a period from 4 to 6 months after being placed on a diet deficient in vitamin A, while control rats on this dietary develop this eye symptom within 30 days. As we have stated in previous papers, certain dosages of x-ray are not different in their action on the system from the ultraviolet ray. It is quite well known at present that the continued administration of ultraviolet to an individual on a ration deficient in vitamin A will enhance the production of vitamin A deficiency. In our studies the same relation is found to exist between the x-ray and these vitamins. The x-ray does not produce or add vitamin B or vitamin A to the system, but it increases their availability or utilization. This would account for the increase in duration of life in direct ratio to the amount of vitamin A added to the dietary. These later studies confirm these findings and our present studies on vitamin A concentrates will be interesting in that they will demonstrate how concentrated a form of vitamin A can be utilized by the system.

Conclusions. Provided a well balanced dietary is given, the destructive action of x-rays on the organism is protected in direct ratio by the concentration of vitamin A added to or available in the animal body.

---

[3] Jorstad, L. H., and Lane, C. W., to be published in *J. Am. Med. Assn.*

4124

Effects of Irradiation of Crude Coal Tar by Quartz Mercury Vapor Lamps. I. Evidence of chemical changes as shown by changes in absorption spectra.

JULIA HERRICK AND CHARLES SHEARD.

From the Section on Physics and Biophysical Research, The Mayo Clinic and The Mayo Foundation, Rochester, Minnesota.

The stimulus to investigations on the possible changes in the absorption of crude coal tar when irradiated by air-cooled quartz mercury vapor lamps was furnished by the fact that a simple but apparently beneficial treatment for psoriasis consists in irradiating, with sources rich in ultraviolet light, affected areas which are covered with a thin film of crude coal tar ointment.[1] Neither the ointment nor irradiation alone produces as marked results; hence the combination of the 2 is used simultaneously. The so-called crude coal tar ointment is made up of 2 drams each of crude coal tar and pulverized zinc oxide mixed with 2 ounces each of corn starch and petrolatum.

It seemed highly probable that the only substance in this ointment which might give evidence of chemical change under ultraviolet irradiation was the crude coal tar and that, by investigations of a spectroscopic character, it might be possible to determine which portion of the crude coal tar was the active or stimulating agent. Thus far we have settled definitely only the question relative to changes in absorption spectra produced by irradiation with an air-cooled quartz mercury vapor lamp. The lamp used was made by the Victor X-Ray Corporation and was operated at 90 volts at a distance of 17 cm.

The apparatus for obtaining absorption spectra is shown in Fig. 1. A 0.5 kilowatt transformer is used for electrical excitation together with a suitable condenser which is joined in parallel (multiple) with a spark-gap consisting of one electrode of aluminum and one of nickel separated by a few millimeters. The photographic plates used for obtaining the spectrograms were made more sensitive to the extreme ultraviolet irradiation by treatment with oil. In the development of the plate the oil is carefully removed with anhydrous ether before it is immersed in the developer. Of the various possible solvents for crude coal tar, ether was chosen because it is least opaque to the ultraviolet region and does not change appreciably (if at all) when exposed to prolonged irradiation. Toluol, benzene, xylol and carbon tetrachloride were discarded because they were too opaque when used with the particular depth of quartz cell (1.346 mm.) employed in these investigations. Chloroform is an excellent solvent for crude coal tar but cannot be used because of the chemical action produced by prolonged irradiation.

---

[1] Goeckerman, W. H., *Northwest Med.*, 1925, xxiv, 229.

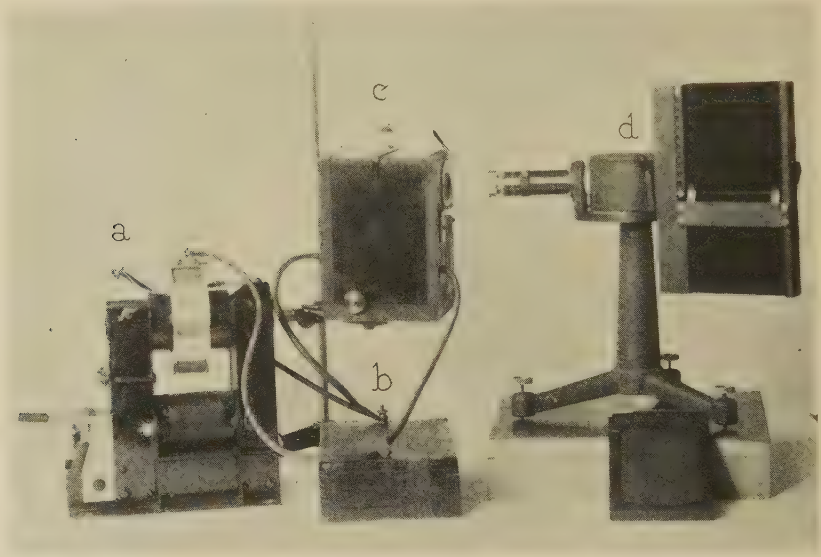

FIG. 1.
Ensemble of apparatus for producing a spark discharge in air and for photographing the spectra in both the visible and the ultraviolet regions: *a*, transformer; *b*, condenser; *c*, spark-gap; *d*, quartz spectrograph.

Spectral changes produced by irradiation of solutions (ether) of crude coal tar. A saturated ether solution of crude coal tar was made and this was diluted by putting 1 cc. of it in 1000 cc. of ether. The diluted solution was put in the quartz cell and carefully sealed. Absorption spectra were taken before and after irradiation. Fig. 2 shows the changes in absorption spectra due to irradiation. Fig. 3 shows the increased spectral absorption of a freshly diluted solution of crude coal tar after standing for periods varying from 20 minutes to 24 hours. Fig. 4 demonstrates that the 24 hour old solution can again be made more transmissive spectrally after successive periods of irradiation. From these and similar spectrograms it is found that the effect of ageing is to increase the absorption. These ageing solutions, however, may be made more transmissive by subsequent ultraviolet irradiation, but the limits and percentages of transmission do not equal those possessed by the original, freshly prepared solution.

Spectral changes produced by the irradiation of crude coal tar (thin film method). In order to avoid any action caused by the presence of a solvent, a thin-film method was employed. Thin films were made by allowing a drop of a 5% benzene solution to fall on a quartz plate.* On the evaporation of the benzene a film of crude coal tar remained, frequently of the order of thickness of 1000 molecules.[2] This film was covered by a second quartz plate and a region of apparently uniform thickness and character care-

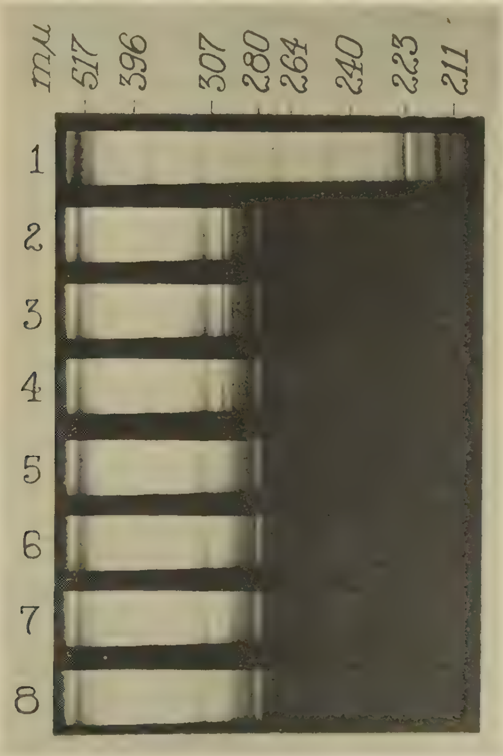

FIG. 2.

1. Spark (air) between electrodes. 2. Quartz cell filled with ether solution of crude coal tar and interposed between the spark-gap and the slit of the spectrograph. 3. Same, 15 minutes later. 4. Same, after a 15 minute irradiation. 5. Same, after an additional 30 minute irradiation. 6. Same, after an additional 60 minute irradiation. 7. Same, after an additional 60 minute irradiation. 8. Same, after an additional 60 minute irradiation. Times of exposure: (1), 20 seconds; (2-8), 60 seconds.

---

* We desire to acknowledge the assistance of Professor Anthony Zeleny and Professor Charles A. Mann of the University of Minnesota in matters pertaining to the technic of the preparation of thin films.

[2] Harkins, W. D., and Feldman, Aaron, *J. Am. Chem. Soc.*, 1922, xliv, 2665.

fully marked off. This enabled us to take successive photographs of the absorption spectra in the same region of the film, so that errors due to nonuniformity of film were obviated. This method presents many difficulties in technic and it is practically impossible to obtain 2 films exactly alike.

Fig. 5 shows a true effect due to irradiation over and above any changes that may occur as the result of ageing. Differences between spectrograms 4 and 5 are due to changes other than irradiation in an interval of 41 hours. Differences between spectrograms 5 and 7 are due to an irradiation of 165 minutes. Spectrograms 5 and 7 were taken immediately after irradiation, so that any changes due to ageing are reduced. Since the differences between spectrograms 5 and 7 are much more marked than those between 4 and 5 and since there is a time interval of only 165 minutes between 5 and 7,

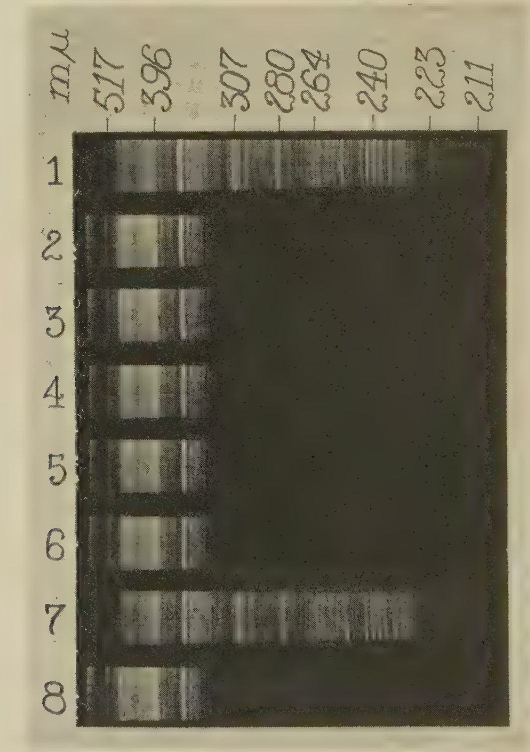

FIG. 3.

1. Spark spectrum. 2. Ether solution of crude coal tar. 3. Same, after 20 minutes. 4. Same, after additional interval of 35 minutes. 5. Same, after additional interval of 60 minutes. 6. Same data as for 5. 7. Spark spectrum. 8. Ether solution following morning (total interval, 24 hours). Times of exposures: (1 to 7), 20 seconds; remainder, 60 seconds.

whereas there is a time interval of 41 hours between 4 and 5, the change is undoubtedly due to the ultraviolet irradiation.

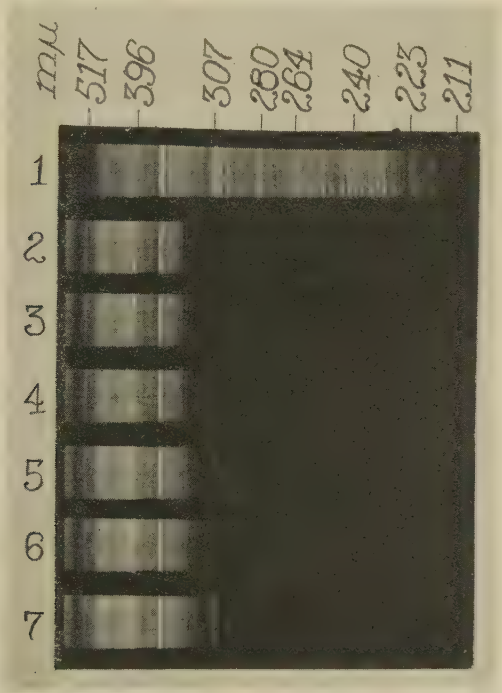

FIG. 4.

1. Spark spectrum. 2. Same as No. 8 in Fig. 3. 3. Same, after 15 minute irradiation. 4. Same, after additional 30 minute irradiation. 5. Same after additional 60 minute irradiation. 6. Same, after additional 60 minute irradiation. 7. Same, after additional 45 minute irradiation. Times of exposures: (1), 20 seconds; (2-7), 60 seconds.

Fig. 6 shows changes in the absorption spectra of a film of crude coal tar (made by the deposition method) both with and without irradiation. There are changes due to ageing. These changes are, however, much less marked than those due to irradiation. The thinner the film the more marked these ageing effects seem to be. Films of crude coal tar, made after the manner of blood smears, were irradiated and then dissolved in ether. These solutions also showed an increased transmission of ultraviolet light produced by irradiation. During irradiation of the crude coal tar, whether in thin films or in solution, special precaution was taken to avoid changes due to heating effects. The substance to be irradiated was placed in an open petri dish on finely chopped ice and a heat-wave filter (consisting of a column of 2 inches of water in contact with a plate of transparent fused quartz) was interposed between the mercury quartz lamp and the dish containing the solution. In all instances the absorption spectra were photographed immediately following irradiation.

It is of interest to compare the results of our investigations with those of Hess and Weinstock[3] on changes in spectral transmission of cholesterol when irradiated by quartz mercury vapor lamps. In many particulars the phenomena observed are the same. The irradiated cholesterol absorbed ultraviolet radiations to a less degree than ordinary cholesterol: an effect which is intensified with increasing degrees of irradiation (within a limited time). By means of suitable filters Hess and Weinstock showed that little if any changes on irradiation are produced by wave lengths longer than 302 millimicrons. With the bare quartz mercury lamps and with sufficient exposure the transmissibility of cholesterol is increased to

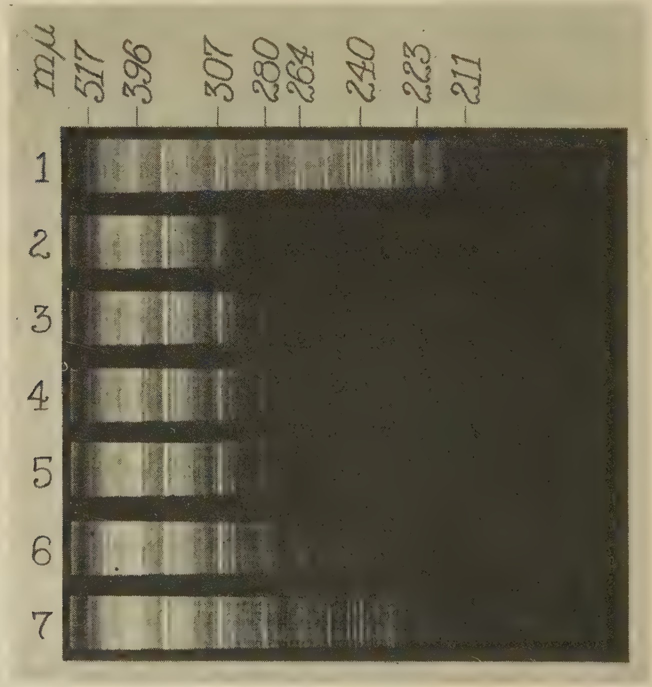

FIG. 5.

1. Spark spectrum. 2. Non-irradiated film of crude coal tar between quartz plates. 3. Same, after 15 minutes irradiation. 4. After additional 30 minute irradiation. 5. After about 41 hours ageing. 6. After an additional 60 minute irradiation. 7. After additional 105 minute irradiation. The total irradiation time of the film was 210 minutes. Times of exposures: (1), 15 seconds; (2-7), 60 seconds.

---

[3] Hess, A. F., and Weinstock, Mildred, *J. Biol. Chem.*, 1925, lxiv, 193.

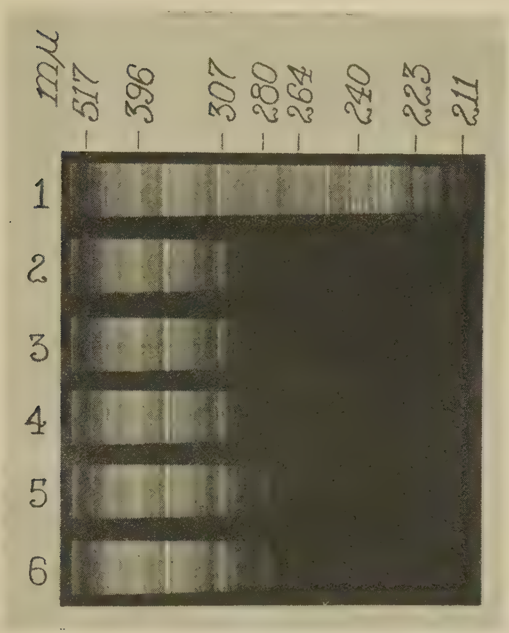

FIG. 6.

1. Spark spectrum. 2. Non-irradiated film. 3. Same, after an interval of 3 hours. 4. After an interval of 18 hours and 30 minutes. 5. Same, after 60 minute irradiation. 6. Same, after additional 60 minute irradiation. Times of exposures: (1), 20 seconds; (2-6), 30 seconds.

a wave length of approximately 256 millimicrons. By increasing the time of irradiation we have found that the transmissibility of crude coal tar is increased to approximately 240 millimicrons for a given concentration. These results naturally raise the question of whether the 2 substances (cholesterol and crude coal tar) do not contain a common ingredient which is responsible for the results obtained. It is possible, although questionable, that cholesterol is present in crude coal tar. At any rate, there is evidence in both cholesterol and crude coal tar of similar chemical changes after irradiation with quartz mercury vapor lamps, as evidenced by changes in transmissibility of ultraviolet radiations. We expect to present further evidence bearing on these points in a later communication.

Conclusions. (1) Ether solutions of crude coal tar show increased transmissibility to ultraviolet light after irradiation by an air-cooled quartz mercury vapor lamp. (2) Ether solutions, on standing, show a decrease in transmissibility. (3) Although an ether solution of crude coal tar is rendered more opaque on standing, it immediately becomes more transparent after irradiation. (4) Thin films of crude coal tar show this increase in transmissibility after irradiation to a more marked degree than does the ether solution of crude coal tar. (5) These changes in transmissibility, detected by changes in the absorption spectra, are regarded as evidences of chemical changes activated by the quartz mercury vapor lamp.

4125

Some Observations on the Urea Concentration Test.

M. M. WINTROBE.

From the Department of Medicine, Tulane University School of Medicine.

Some observations on the phenolsulphonephthalein excretion and urea concentration of the urine in 49 students are here briefly reported. The urea concentration test was carried out entirely as described by MacLean and de Wesselow.[1] The phenolsulphonephthalein tests were carried out in the usual manner, the dye being injected intravenously in all cases and colors matched without the aid of a colorimeter. All the students examined were normal, healthy adults between the ages of 20 and 30. Urinalysis was negative in all cases.

The maximum urea concentration of the urine varied from 2.4% to 4.1%. In 45 of 49 students the concentration varied from 2.5% to 4.0%. The average was 3.22%. Phenolsulphonephthalein excretion varied from 43% to 82.5% with an average of 66.3%.

When the results of the 2 tests in each case are compared a great variation is noted. Curves of phenolsulphonephthalein excretion and the corresponding maximum urea concentration (with the individual determinations arranged in the order of the phenolsulphonephthalein excretion), do not coincide, as they should if they were both quantitative indicators of the excreting power of the kidneys.

In comparing the results of these 2 tests, it must, of course, be remembered that whereas in the dye test the total amount of that dye excreted in 2 hours is measured, in the second test the concentration of urea in a *varying amount* of urine is determined. This fact is recognized in MacLean's instructions that specimens of 120 cc. or over should be discarded as the dilution of the urea is in such cases too great to be reliable. Curves of volume of urine, plotted in relation to the corresponding urea concentration, indicate that the amount of urine, even when only quantities less than 120 cc. are considered, influences the reading more than has heretofore been recognized. Urea concentration rises and falls very definitely with the volume of urine in which the urea is dissolved. This obviously affects the value of the urea concentration test as a quantitative indicator of renal function.

---

[1] MacLean, H., and de Wesselow, O. L. V., *Brit. J. Exp. Path.*, **1920, i, 53.**

Again since one test is concerned with the excretion of a dye totally foreign to the body, while the other is concerned with the end product of protein metabolism normally found in the body, another factor which may influence the urea concentration reading is the amount of urea normally being excreted by the individual at the time of the test. Some idea of this can be gained by determining the concentration of urea in the urine just before the test. This was done in 38 students. Concentration varied from 0.6% to 3.4%, with an average of 1.49%. There appeared to be some relation between concentration of urea prior to the test and urea concentration 2 hours following the taking of 15 gm. of urea by mouth.

Whether by some mathematical computation the above causes of variation in the urea concentration can be allowed for and the test thus made a more quantitative measure of renal function, remains to be seen.

In one respect the tests have been quite consistent. In practically all cases the urea concentration was 2.5% or more. Likewise, phenolsulphonephthalein excretion was 40% or over in every case, and in 46 of 49 cases was above 50% at the end of 2 hours. Both tests were consistent, therefore, in indicating a healthy state of the kidneys.

4126

An Example of Bacterial Synergism on Endo Medium.

T. F. NICHOLSON. (Introduced by W. L. Holman.)

From the Department of Pathology and Bacteriology, University of Toronto.

Bacterial Synergism has usually been studied in fluid medium. I wish to report here an example of the association of *B. welchii* and *B. faecalis alkaligenes* on an Endo plate with a resulting change of the type of colony formed by the latter. A swab taken from the drainage wound of an acutely infected shoulder, was planted in hormone-dextrose-broth and on blood agar. The broth showed a growth of gram negative bacilli and gram positive cocci in chains. The blood agar showed haemolytic colonies of streptococci and colonies of the coliform type. The broth culture was spread on an Endo plate and after 24 hours incubation this plate showed numerous colonies with red centers and narrow, colorless peripheries. In such isolations only colorless colonies are usually picked for further study, but because of the colorless periphery of these colonies, one was transferred to hormone broth. A smear from a 24 hour growth of this culture showed nothing but gram negative bacilli. Generous portions of the broth culture were transferred to tubes of broth containing dextrose, lactose, saccharose and salicin and to litmus milk. After 24 hours there was fermentation with the production of acid and gas in the tubes with dextrose, lactose and saccharose and the typical "stormy fermentation" of *B. welchii* in the litmus milk. Gram negative and gram positive bacilli were seen together in all the tubes, except that containing salicin.

The two organisms were isolated in pure culture. The gram negative bacillus fermented none of the carbohydrates and gave an alkaline reaction in litmus milk. The gram positive bacillus gave the typical reactions of *B. welchii* in litmus milk and grew well in Veillon agar and on blood agar plates in an anaerobic jar. It was also pathogenic for guinea pigs. The *B. faecalis alkaligenes* produced colorless colonies on Endo agar. The *B. welchii* did not grow on this medium under aerobic conditions. Mixtures of the two planted on Endo agar reproduced the red centered colony with the colorless periphery as seen in the original plate. The anaerobe apparently grows in the interior of the colony of the aerobe and here acts on the lactose and produces acid. Two other examples of this phenomenon were noted later, one from a sample of faeces from a case of suspected typhoid and another from a swab from an infected appendix.

The possibility of this association between *B. welchii* and non-lactose fermenting coliform organisms causing confusion and mistakes in the detection of *B. typhosus* is one which should be seriously considered. Moreover, in detecting *B. coli* by presumptive test it is usual to plate on Endo medium from the positive lactose tube, red colonies being considered as evidence of the presence of *B. coli*. An interesting point in my finding was that the anaerobe remained in the mixture after growing; in an open tube of dextrose-hormone-broth; in surface colonies on Endo agar; in another hormone-broth and in transfers from this to a series of aerobic culture media.

4127

Effect of Cholesterol Activated by Ultra-violet Irradiation on Growth of Tubercle Bacilli in Vitro.

ALAN R. MORITZ. (Introduced by Harry Goldblatt.)

From the Department of Pathology of Lakeside Hospital and the Western Reserve School of Medicine.

The beneficial effects of ultra violet light in the treatment of tuberculosis have been known since the pioneer work of Finsen (Bie).[1] More recently the curative effect in intestinal tuberculosis has attracted attention. Experimental proof of the nature of this action, whether a general constitutional effect or a specific inhibitory influence on the growth of the organisms has not been offered.

The bactericidal action of direct ultra-violet irradiation is well known. Eidinow[2] states that it has been shown that an "erythema dose" of ultra violet light applied to the skin of animals increased the hemo-bactericidal power of the blood. Ultra violet light then appears to have both a direct and an indirect bactericidal action, but whether this bactericidal action is possessed by irradiated photosensitive substances is not known. It has been shown that cholesterol irradiated by ultraviolet light and ingested produces some of the biological effects which are produced by direct irradiation. More recently the effect of the cholesterol has been attributed to a contamination, ergosterol, which is usually present in unpurified cholesterol.

It seemed of interest to test the effect of biologically active (irradiated) impure cholesterol upon the growth of tubercle bacilli *in vitro.*

Experimental: Cholesterol (Merck) was biologically activated by exposure to ultra violet light for one hour at a distance of 50 cm. The source of ultra violet light was a Hanovia, mercury vapor quartz tube. The cholesterol was dissolved in ether, poured into flat dishes and allowed to dry. In this manner a thin film of cholesterol was exposed to the light. This cholesterol was shown to be biologically active because 15 mg. daily prevented the development of rickets in 3 rats on a rickets-producing diet (Steenbock No. 2965), while 3 rats on the same diet that received the same amount of non-irradiated cholesterol developed severe rickets (histological).

Culture medium was then prepared to test the effects of the biologically activated cholesterol on the growth of tubercle bacilli *in vitro.* Twelve tubes of glycerine agar containing a 0.5% suspension of irradiated cholesterol and 12 tubes containing a 0.5% suspension of non-irradiated cholesterol were prepared. The cholesterol suspension was obtained by dissolving the required amount of cholesterol in ether and vigorously stirring the ether suspension into the hot medium. In this manner a finely dispersed suspension was obtained and the medium had a milky opalescent appearance. Each of the 24 tubes was then inoculated with human tubercle bacilli. The tubercle bacilli were obtained from the American Type Culture Collection. Inoculation of the glycerine agar tubes was made evenly by suspending the tubercle bacilli in a small amount of normal saline and inoculating the culture medium with equal amounts by means of a capillary pipette. The tubes were then plugged, corked, incubated and examined at 7-day intervals up to 4 weeks. A luxuriant growth of tubercle bacilli was obtained on all tubes inoculated save one which was overgrown by contaminating yeast. There was no appreciable difference in either rate or amount of growth between those cultures growing on medium containing a suspension of non-irradiated and those growing in medium containing a suspension of irradiated cholesterol.

---

[1] Bie, U., *Brit. Med. J.*, 1899, ii, 825.

[2] Eidinow, Albert, *Proc. Roy. Soc. Med.*, 1926, xix, 50.

A culture medium was then employed in which the cholesterol was in solution rather than in suspension. For this purpose Dorset's egg medium was chosen and 24 tubes were prepared as follows: Twelve tubes were filled with ordinary egg medium and 12 with egg medium which had previously been irradiated for one hour at a distance of 50 cm. from the lamp. These 24 tubes were inoculated and incubated in the same manner as the glycerine agar tubes and again no appreciable difference was observed in either the rate or the amount of growth at the end of 4 weeks' incubation.

Conclusions: The growth of human tubercle bacilli was not affected by a medium containing a suspension of cholesterol irradiated by ultra violet light or by an irradiated medium containing cholesterol in solution.

4128

Development of Vitamin A During Ripening of Tomatoes.

AGNES FAY MORGAN AND LAURA LEE W. SMITH.
(Introduced by Carl L. A. Schmidt.)
From the Laboratory of Household Science, University of California.

In the progress of an inquiry into the conditions under which vitamin A is developed in plants a comparison was made of the vitamin

A content of tomatoes ripened under various conditions. The tomatoes were of the variety called San Jose Canner and were supplied us by J. T. Rosa of the University Farm, Davis, California. They were plucked from the vines when green but completely matured, and were divided into 6 lots. One lot, designated lot 1, was ground at once, placed in small air tight glass jars fitted with outlet and inlet glass tubes, the air from which was evacuated and released with carbon dioxide 4 or 5 times. The jars were then stored at 17 degrees below zero centigrade. This process of preparation and storage was used to ensure as much freedom from oxidation as possible during the testing period. The other lots of tomatoes were similarly treated after being ripened as follows: lot 2, ripened in diffused light; lot 3, ripened in the dark; lot 4, ripened in ethylene, 1:2000 parts for 1 week[1]; lot 5, ripened in the dark except for 3 periods of ultra-violet irradiation of 30 minutes each at 12 inches distance from the arc. Temperature, ventilation and humidity were kept constant during ripening for all lots, a precaution indicated by the work of Duggar.[2] In addition lot 6 was later taken from the vines in the ripened condition. An attempt was made to allow all specimens of the fruit to ripen to a uniform color by comparison with Tallquist hemoglobin standards, but the results were not satisfactory since all the tomatoes with the exception of lot 4, showed varied degrees of redness in different parts of the same fruit. The ethylene-ripened specimens, lot 4, were uniformly colored a deep red, the tint of the deepest colored portions of the other fruits. The ripening period occupied 10 days in diffused light, 19 days in the dark, 7 days in ethylene, and 27 days for the ultra-violet treated lot. The oxidase contents of all specimens were determined but these were found to exhibit no significant differences.

Vitamin A deficient young rats were used for testing the value of these preparations by feeding separately carefully weighed portions of the fruit in addition to 0.5 gm. yeast daily and the usual basal diet. All rats used were taken from mothers reared and maintained on identical diet, were placed on this basal diet at 21 days of age and given the curative doses of tomato only after eye disease and loss of weight developed after 30 to 40 days. The basal diet consists of baked and alcohol-extracted casein, 18 parts; irradiated crisco, 5; agar, 2; salt mixture (Osborne and Mendel), 4; dextrin, 71. The feeding period was 56 days, and an attempt made to determine the dosage which allowed 6 to 8 gm. increase in weight weekly. This standard was adopted rather than that of 3 gm. increase weekly proposed by Sherman and Munsell,[3] because the rats maintained at the former level are so often rendered abnormal by intercurrent and accidental results of their extremely low vitamin A margin.

---

[1] Rosa, J. T., *Proc. Am. Soc. Hort. Sci.*, 1926.

[2] Duggar, B. M., "Lycopersicin, the red pigment of tomatoes, and the effect of conditions upon its development." *Wash. Univ. Studies*, I, 1913.

The results of the test shown in Table I indicate the relative poverty as to vitamin A of the green tomatoes, and its consistent development in the ripened fruit. This is somewhat unexpected in view of the usual occurrence of this vitamin in green plant products and of the recent work of Dye, Medlock and Crist[4] in which the occurrence and production of chlorophyll and of vitamin A together has been indicated. As the lycopin developed in the plucked tomatoes as well as in those ripening on the vines apparently vitamin A developed also. This would seem to indicate a local production of the vitamin in the fruit itself. Certain findings upon the increase in

TABLE I.

Vitamin A Content of Tomatoes, Green and Ripened under Various Conditions.

Lot No.	Method of preparation	Amount fed daily	No. of animals	Average gain in 8 weeks or expt'l period	Remarks.
		gm.		gm.	
1	Green. pH, 4.86	0.10	2	—	Average survival, 42 days.
		0.20	4	14	2 died, av. survival, 69 days.
		0.30	3	–5	All died, av. survival, 54 dys.
2	Ripened in diffused light pH, 5.21	0.00	1	–4	Died on 35th day.
		0.10	4	33	1 died on 77th day, 3 killed.
		0.20	2	59	Very good condition.
3	Ripened in the dark. pH, 4.75	0.00	1	–2	Died on 52nd day.
		0.10	3	16	Average survival, 80 days. 2 died, 1 killed.
		0.20	2	73	Good condition.
		0.30	1	86	Very good condition.
4	Ripened in ethylene 1:2000 for 1 week. pH 5.04	0.00	3	–4	Died in 34 days.
		0.10	3	36	Fair condition.
		0.20	2	59	Good condition.
		0.30	2	90	Very good condition.
5	Ripened in dark except for 90 min. ultraviolet irradiation. pH, 4.88	0.00	1	–4	Died on 35th day.
		0.10	2	4	Died, av. survival, 63 days.
			3	47	Poor condition.
		0.20	2	58	Good condition.
6	Ripened on the vine. pH, 4.66	0.00	2	—	Died in 45 days.
		0.10	5	35	Poor condition.
		0.20	2	59	Fair condition.
		0.30	2	66	Very good condition.

---

[3] Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 1925, xlvii, 1639.

[4] Dye, M., Medlock, O. C., and Crist, J. W., *J. Biol. Chem.*, 1927, lxxiv, 95.

vitamin A in red bell peppers as compared with the green variety from which they develop after plucking from the vines which were observed in this laboratory several years ago, parallel this experiment.

The vine ripened tomatoes and 4 examples of the fruit ripened off the vines under various light conditions appear to be about equally rich in vitamin A. Some interest attaches to the adequacy of the ethylene ripening process in this connection.

We are now engaged in determining the quantitative relations of the fat-soluble pigments, carotin, xanthophyll, and lycopin with chlorophyll and vitamin A in tomatoes and other plant products when these have been exposed to the action of ultra-violet and other light rays. The curious delay in ripening which was encountered in the ultra-violet treated lot number 5, may have been caused by some injurious action of the ultra-violet light, possibly through stimulated autoxidation, such as Holm, Greenbank and Deysher[5] have shown for unsaturated oils.

A protecting effect of carotin and lycopin in allowing vitamin A production in plants when these are exposed to sunlight is suggested as an explanation for the consistent occurrence, and as here shown development, of vitamin A along with one or the other or both of these plant pigments. The work of Coward[6] and of Heller[7] on the production of vitamin A in etiolated and normal wheat shoots under light treatment is not inconsistent with this view, although their choice of seeds and seedlings normally poor in both carotinoid pigments and vitamin A makes interpretation of their data difficult. The effect of changes which accompany germination in the growing plant and in the rate of growth of the plant as influenced by amount and kind of light can not be separated from the changes in vitamin A formation recorded in their experiments. The tomato ripening tests here recorded are independent of the changes involved in plant development although a definite metabolism in the plucked fruit must be postulated.

---

[5] Holm, G. E., Greenbank, G. R., and Deysher, E. F., *Ind. and Eng. Chem.*, 1927, xix, 156.

[6] Coward, K. H., *J. Biol. Chem.*, 1927, lxxii, 781.

[7] Heller, V. G., *J. Biol. Chem.*, 1928, lxxvi, 499.

4129

Hypothermia Due to Localized Lesions in Midbrain of Rabbit.

LILLIAN M. MOORE.

From the Department of Physiology, University of California.

These lesions were produced by injections of chromic acid under ether or urethane anesthesia and subsequent gross and microscopic studies made of the brain structures injured. Lesions in one limited area were always followed by a gradual fall in body temperature from a normal of 38° or 39° to 28° or 26° C. within 3 to 5 hours. Injection at any other point so far studied was without marked temperature effect. Accompanying the hypothermia was a gradual decrease in muscular tonus and at 26° C. almost complete atonia. The animal in this condition appears lifeless but if properly cared for can be kept for a number of days.

These results were repeatedly obtained by the injections (some 100) which have been made to date. The microscopic studies are at present in progress.

From the brains already examined it appears that the effective lesion is in the central portion of the superior quadrigemina, 2 to 3 mm. from the aqueduct and within a few millimeters of (but never including) the red nucleus. Since the puncture may be made from a dorsal or lateral direction with the same effect, the superficial midbrain structures are excluded. Within the line of injury are the central gray matter and several nuclei and fiber tracts, notably the nuclei of the third and fourth cranial nerves, portions of the superior cerebellar peduncle, and the posterior longitudinal bundle. It is impossible as yet to say which of these when injured is responsible for the hypothermia or hypotonus resulting, but I feel reasonably sure that some or all of the fibers in the posterior longitudinal bundle are involved in every typical case. This bundle is made up largely of ascending fibers from the vestibular nuclei (probably all 3 nuclei) which are commonly agreed to be necessary for the maintenance of normal tonus. Dysfunction of these nuclei always causes atonia perhaps by removal of an inhibition to the normal myotatic reflex inhibition of the muscle itself. The bundle also includes descending fibers from the interstitial and other less known nuclei of the hypothalamus.

The explanation of the hypothermia which suggests itself is that it is not due to dysfunction of a hypothetical temperature center but that it is an indirect result of the abnormally lowered tonus of all the musculature of the body, an atonia sufficient to decrease appreciably the heat production. Normal temperature would, therefore, depend on the function of a central neural mechanism, probably the vestibular nuclear apparatus, responsible for normal muscular tonus and for normal heat production.

4130

Experimental Paratyphoid Intoxication in Man.

GAIL M. DACK, WILLIAM E. CARY AND PAUL H. HARMON.

From the Department of Hygiene and Bacteriology, University of Chicago.

In a number of food poisoning outbreaks competent bacterial examination has failed to reveal the presence of any living microorganisms. It has been inferred especially by Savage and White[1] that many of the outbreaks were due to poisonous thermostabile growth products of *B. aertrycke* or *B. enteritidis*. Animal experimentation along this line has not met with uniform success since most workers have been unable to reproduce symptoms of paratyphoid intoxication by feeding the sterile products of *B. aertrycke* or *B. enteritidis* to animals.

In our experiments 24 people, ranging in ages from 20 to 40 were fed heat killed cultures or filtrates of *B. aertrycke* and *B. enteritidis*. Nine strains were used, 5 of *B. aertrycke* and 4 of *B. enteritidis*. The date of isolation and colony morphology of these strains are as follows:

Strain No.*	Year	Colony Morphology†	Strain No.*	Year	Colony Morphology†
E 520	1888	R, I, S	Ae388	1922	S
Ae522	1892	R	Ae391	1922	S
Ae518	1898	R	Ae411	1923 (Apr.)	S
E 396	1921	I, R	E 438	1924 (Dec.)	S, I
E 397	1921 (Oct.)	I, R			

* Ae prefix refers to *B. aertrycke*. E prefix refers to *B. enteritidis*.

† R=rough, I=intermediate, S=smooth. Arranged in order of predominance.

The cultures were grown in beef heart medium containing 1% of dextrose. They were incubated at 37° C. for periods varying from 2 hours to 20 days. After incubation the supernatant fluid from each culture was divided and half was filtered through a Mandler filter and the remaining half was boiled for 20 minutes. Before feeding both the heat killed cultures and filtrates were tested for sterility. Rabbits were given intravenous injections of the heat killed cultures and filtrates in amounts ranging from 0.5 to 2.0 cc. All of the animals showed symptoms of dyspnea and many showed prostration and death. The filtrates from the 6 hour cultures were much more toxic than those from older cultures.

---

[1] Medical Research Council, Special Report Series, numbers 91 and 92, London, 1925. Published by his Majesty's Stationery Office.

Human subjects were fed amounts varying from 20 cc. to 340 cc. of heat killed cultures or filtrates. No symptoms developed in any instance. In order to obtain a freshly isolated strain we injected 2 cc. of a veal infusion broth culture of Ae411 into the gall bladder of a *Macacus rhesus* monkey. The animal died after 8 days and the organism was recovered from the gall bladder. This strain was grown in beef heart dextrose medium for 10 days. One hundred cc. of the culture, after boiling for 20 minutes, was then fed to a human subject. No symptoms developed in this individual. Fecal examinations for members of the paratyphoid group of organisms were made on all of our subjects and in no case were we able to isolate paratyphoid organisms. Twenty of the 24 human subjects had been previously vaccinated for typhoid; the remaining 4 had never been vaccinated. Macroscopic agglutination tests were made, using the serum of the human subjects against the homologus organisms which they were fed. These tests were made before feeding and 10 days after feeding. In no case were the agglutination titres increased after feeding.

Conclusions: Heat killed dextrose beef heart cultures and filtrates of 5 strains of *B. aertrycke* and 4 strains of *B. enteritidis* when fed to adults on an empty stomach failed to produce symptoms of paratyphoid intoxication. No agglutinins for the homologous strains were present in the serum of these subjects 10 days after feeding.

4131

A Peculiar Reaction (Allergic?) to Scarlet Fever Streptococcus Toxin.

MARTIN FROBISHER, JR.

From the Department of Pathology and Bacteriology, the Johns Hopkins University.

During the winter of 1925-26 studies on scarlet fever toxin necessitated the frequent intracutaneous injection of various toxic filtrates. The author served as a subject for these tests and until December 9th, 1927, always gave a typical Dick reaction. On this date, however, and on every subsequent test he gave the peculiar, two-phase reaction which is described below. On this occasion there appeared, within 15 minutes after injection, a raised, firm, whitish or cream colored spot about 2 cm. in diameter with a blotchy red or pink edge, the whole strongly suggesting a serum wheal in an anaphylactic person. This disappeared in about 2 hours. Four hours later the typical, red, Dick reaction began to appear. Five days later a toxin which had been prepared some months previously from a different lot of broth but with the same strain of streptococcus was diluted and injected as usual. This toxin had always previously given a typical Dick reaction, the first signs of which did not begin to appear for 6 to 8 hours after the injection. In this instance, within 2 hours, a diamond shaped wheal, 1.5x2 cm. in size, raised, sharply demarked, tender, itching and so yellow in color as to suggest suppuration appeared. There was little or no erythema. This gradually subsided. Several hours later this was completely overspread by an intensely red and somewhat swollen area which finally became an oval of 4x6 cm. at the end of 24 hours.

Some of the same material was injected into the shoulder, all previous injections having been made in the flexor surface of the forearm. The reactions obtained were like those previously obtained but were smaller. Their yellow color was no less marked, however. Neither of the 2 lots of sterile broth gave any reaction even when injected undiluted.

Filtrates from other strains of scarlet fever streptococci were tested. Some gave the early, yellow phase (A) and some did not. It was found that the intensity of the "A" phase, but not its time of appearance, seemed to parallel the intensity of the "B" phase. In other words it seemed to parallel the presence of toxin. In this connection the 24 hour growth on 4 agar plates was washed 3 times in saline solution, suspended in broth, heated for 1 hour at 60° C, 0.25% trikresol was added and some of the suspension injected as usual. No reaction occurred. It is recognized that the heating may have changed the bacterial protein immunologically.

It is suggested that this peculiar reaction may represent a state of allergy toward the streptococcus toxin resulting from the repeated injections. In this allergic condition the filtrate appears to elicit an early reaction (A) due to the presence of the foreign protein *per se* and a late reaction (B), (the true Dick reaction) because of its toxic properties. The 2 reactions are quite distinct in appearance and in development time. They still appear with full intensity and have done so upon each injection since first observed. Other persons sensitive to the toxin tested at the same times and with the same filtrates have not been observed to give the (A) reaction. It is suggested that this sort of reaction might give some insight into the rôle of allergy in scarlet fever.

4132

Ciliary Currents in Oviducts of Turtles in Relation to Transportation of Spermatozoa.

G. H. PARKER.

From the Zoological Laboratory, Harvard University.

The oviduct of the painted turtle, *Chrysemys picta* (Schneider), measures about 15 cm. in length. The proximal two-thirds of it are concerned with the formation of the egg albumen, and the distal third with the production of the egg shell. The whole interior of the duct is ciliated. These cilia form 2 systems, one that covers most of the inner face of the duct and whose direction of stroke is toward the oviducal outlet—that is, away from the ovary (abovarian system), and a restricted system in the form of a narrow tract extending throughout the length of the duct with cilia beating toward the ovary (proövarian system). Living spermatozoa can make no headway up the oviduct against the beat of the abovarian cilia. Hence there is no ground for assuming that rheotaxis plays any part in the migration of the sperm. Neither does the muscular activity in the oviducal wall nor ciliary reversal contribute to this end. The spermatozoa are apparently transported from their region of deposit by the male near the oviducal outlet to the neighborhood of the ovary solely by the system of proövarian cilia, and this transportation of the sperm is doubtless the function of the proövarian cilia. The abovarian cilia keep the oviduct clear and help in part to move the eggs. Whether a proövarian system of cilia occurs in other reptiles than turtles, in birds or in mammals remains to be ascertained.

4133

An Automatic Drop Recorder.

M. S. BISKIND AND MORRIS DAN. (Introduced by Torald Sollmann.)

From the Department of Pharmacology, School of Medicine, Western Reserve University.

This drop recorder was designed to meet the requirements of perfusion and excretion experiments where a continuous record of outflow is desired. It differs in principle from the mechanical type of drop recorder in that the conductivity of the solution itself is employed to close the electrical circuit, thus avoiding completely the uncertain response produced by impact of the falling drops with a weighted or spring lever. It operates with solutions containing electrolytes even in low concentrations, *f. i.*, with Cleveland tap water, but it is not suitable for use with distilled water.

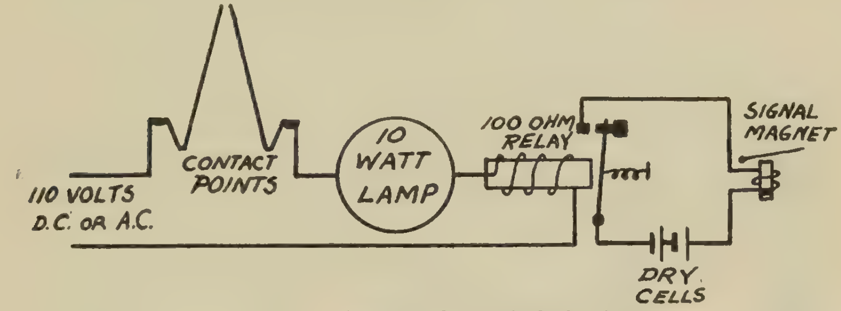

Fig. 1. Diagram of electrical circuit.

Fig. 1 illustrates the electrical circuit. The drops, as they pass the tips of 2 contact points, close a primary circuit which works a 100 ohm "pony" relay (*f. i.*, Cat. No. 573, J. H. Bunnel and Co., New York). A signal magnet is activated through the relay by 2 dry cells.

The relay is best operated by a direct current of 110 volts; but alternating current may be used if the height from which the drops fall is so adjusted that the latter make contact at the points for a time which is less than a half-cycle of the current (usually 1/120th second). A fall of 3 to 4 cm. will be found satisfactory if the points are properly adjusted. The 10 watt lamp is placed in the primary circuit to protect the relay in case the points should accidentally become short-circuited. If the solution used contains electrolytes equivalent to 0.9% NaCl or over, a 22½-volt current (from a radio "B" battery) may be used instead of the 110-volt current (in which case the lamp may be omitted) but the use of 110 volts as standard affords an apparatus of wide adaptability.

Fig. 2 shows a convenient holder for the contact points. The points (1) which are preferably of platinum wire (0.020 in. dia., No. 25 B & S gauge) are fixed by 2 machine screws in a bakelite shell (2) and are connected by means of insulated No. 18 copper wire through a brass tube (4) of optional length (*f. i.*, 5 cm.) to 2 binding posts (5) set in a bakelite disc on the end of the brass tube.

It is important that the points (1) be bent as shown in the diagram with the tips about 1 mm. apart; otherwise the fluid may produce a permanent short-circuit either at the tips or around the base

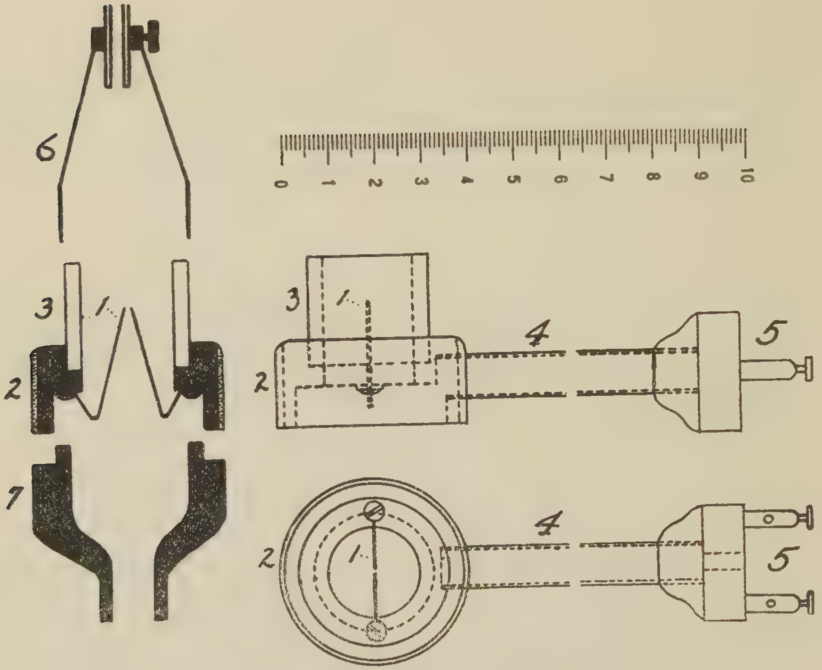

FIG. 2. Holder for contact points. Scale in millimeters.

of the shell (2). An aluminum sleeve is fitted tightly around the bakelite shell (2) to prevent splitting. All exposed metal on the under side must be covered with sealing wax or other insulating material. Into the top of the shell is fitted a glass tube (3) (from No. 000 Lunkenheimer oil cup) which serves to protect the points against accident. (The points may be easily adjusted from the bottom of the shell.) Above the glass tube is a support (6) which fits snugly over the tube (3) and serves to center and hold rigid the tube from which the drops originate. It consists of a split brass ring, to which is attached by means of 2 or 3 strips of 5 mm. spring brass a small collar, drilled to hold the "dropping tube" by means of a set screw. This comes to about the correct height for use with 60 cycle alternating current. Below the shell (2) is a bakelite drainage cup (7) which fits tightly into the shell and serves to drain the fluid into a rubber tube connected to the cup. A funnel may be used in its place. The brass tube (4) is used as a support for clamping the holder to a stand.

In practice, the lamp, relay and 2 dry cells (Fig. 1) are conveniently enclosed in a box 6x6x7 inches having appropriate binding posts for connection to the source of current, the contact points and the signal magnet.

During class demonstration of perfusion or excretion experiments, it has been found that the sound of the relay is a valuable adjunct to the visual record made by the signal magnet. If the relay itself does not click loudly enough, a telegraph sounder may be incorporated in the secondary circuit. Changes in rate of flow are easily impressed on the class by this method.

By using a signal magnet wound to 1000 ohms, the relay and lamp may be omitted from the circuit and the contact points connected directly in series with the signal magnet, using a 22½-volt B battery as the source of current. This latter circuit is not advised for general use inasmuch as it requires a specially made and rather delicate signal magnet. This can be made in the style of the Harvard signal magnet from the magnets of a radio headphone, substituting for the permanent horseshoe magnet of the phone a short bar of soft iron.

4134

Variations in Monocytic Response to Peroxydase Test.

F. A. McJUNKIN.

From the Department of Pathology, Loyola University School of Medicine.

Naegeli[1] states that almost all, if not all, of the human monocytes are peroxydase reacting. The author[2] found no peroxydase-reacting monocytes in the blood of a rabbit. In a normal adult man there were present 3.4% peroxydase mononuclear phagocytes and 1.1% phagocytes that did not react to the peroxydase test. The conclusion was that the peroxydase cells of human blood are of myeloid origin, which is the view held by Naegeli. The negative phagocytes were thought to be the true monocytes derived from the reticular portion of lymphoid tissue. Recently some unpublished observations have brought me to a consideration of the alternative view, namely, that the peroxydase-positive phagocyte gets its granulation secondarily in a more or less accidental manner. The conclusions arrived at in this paper are based on tissue cultures of blood leukocytes.

Methods and Results: Numerous cultures of human blood were made by the coverglass method and examined by the peroxydase test daily from one day to 4 weeks. This was done on the assumption that the peroxydase granulation of the monocytes would disappear, provided the granulation was acquired secondarily and was not an essential part of the cell cytoplasm. After one month in the incubator large peroxydase reacting phagocytes were present in the cultures. Never was the number of phagocytes great and no conclusion was possible in regard to the proportion of positive and negative cells. Rhoads and Parker[3] found at the end of 2 weeks of incubation both positive and negative mononuclear phagocytes in normal human blood. The next procedure was to culture rabbit leukocytes in contact with variable amounts of human blood: Rabbit blood was cultured on sterile cover-glasses covered with smears of human blood made with aseptic precautions. The smears were kept dry in the air for 2 hours. The rabbit blood was obtained from the heart and drops of blood placed directly on the covers from the small caliber needle. The covers which had previously been rimmed with sterile vaseline were inverted over the well and placed in the incubator within 5 minutes after withdrawal of the blood from the heart. After about one hour the covers were sealed permanently with paraffin. At the end of 2 weeks as many as 500 large mononucleated cells were present in some of the cultures while in the human blood cultures mentioned above the cells at the most numbered a few dozen. Hundreds of the rabbit blood cultures were examined by the peroxydase method after 1 to 21 days of incubation: cover-glasses removed and covered for 30 seconds with 2 drops of benzidin (100 mgm. of dry benzidin, 20 cc. pure methyl alcohol, 5 cc. distilled water, 1 drop hydrogen peroxide) and then diluted with 4 drops of 1/10% copper sulphate. After a reacting time of about 2 minutes the cover is washed in distilled water made slightly acid with acetic acid, stained in hematoxylin (Harris) for one minute and again washed in the acidulated water in order to color the nuclei a purple so as to contrast with the green peroxydase granules. The preparations are blotted, dehydrated with acetone, cleared in xylol and mounted in balsam. The method is one used by the author[4] since 1920 except the dilute copper sulphate is substituted for distilled water for dilution. Copper sulphate was recommended by Sato and Yoshimatsu.[5]

---

[1] Naegeli, O., *Blutkranheiten und Blutdiagnostic,* Berlin, 1923.

[2] McJunkin, F. A., *Arch. Int. Med.,* 1923, xxxvi, 799.

Although in the usual smear of human blood kept dry at room temperatures for 2 or 3 days the peroxydase reaction becomes greatly diminished it is well retained for 5 days in the cultures in the parts of the film not covered by the droplets of rabbit blood. In fact, the neutrophilic granules swell to become more conspicuous than in the fresh dry smear. By the twelfth day the granules have become indistinct. The best preparations have been obtained after 5 to 6 days of incubation. At this time there may be present almost as many large mononuclear cells in the peripheral zone as there are rabbit polynuclears. Many of them contain phagocyted nuclear material and in an occasional rabbit phagocyte there are peroxydase particles permitting certain identification. The greenish color of the granules makes possible a differentiation from nuclear particles and from hemoglobin. Sometimes the granules correspond in size to those of the human neutrophile while others have been seen in which the reacting cytoplasm was in a mass suggesting a portion of phagocyted human neutrophile. Ameboid rabbit monocytes have been seen in contact with human polynuclears. The presence of phagocyted material in the cells serves to eliminate a possible confusion of rabbit monocytes with persisting human monocytes. The abundant hemoglobin becomes even more objectionable after formalin fixation. Work is now in progress to culture the rabbit phagocytes in plasma with elimination of the rabbit red cells.

---

[3] Rhoads, C. P., and Parker, F., *Am. J. Path.*, 1928, iv, 271.

[4] McJunkin, F. A., *J. Am. Med. Assn.*, 1920, lxxiv, 17.

[5] Sato, A., and Yoshimatsu, S., *Am. J. Dis. Child.*, 1925, xxix, 301.

The author wishes to express his indebtedness to Mr. Anthony J. Bell for assistance in the earlier experiments.

Conclusion: Rabbit monocytes negative to the peroxydase test may acquire a positive reaction when cultured on smears of dry human blood. Since rabbit monocytes in tissue cultures may by phagocytosis acquire a peroxydase content it seems possible that the mononuclear phagocytes of human blood may get their peroxydase granules by the same process.

4135

Magnesium Sulfate as a Factor in Retention of Calcium and Phosphorus in Cattle.*

L. S. PALMER, C. H. ECKLES AND D. J. SCHUTTE.†
(With the technical assistance of W. M. Neal and T. W. Gullickson.)

From the Divisions of Agricultural Biochemistry and Dairy Husbandry, University of Minnesota, St. Paul.

We[1] reported in connection with a study of a P deficiency in the rations of cattle in parts of Minnesota that the drinking water in the affected regions contains an unusually large amount of magnesium sulfate. This was suspected of being a factor in the mineral deficiency problem because of the well-known antagonism between Mg and Ca, and indirectly because of the close relationship between Ca and P in metabolism. It was shown that aphosphorosis could be produced experimentally in dairy cattle during liberal feeding of prairie hay and oats grown in the affected regions. The diseased condition could be both prevented and allayed by a P supplement in the form of $Ca_3(PO_4)_2$, bonemeal or NaH_2PO_4; under the latter conditions the feeding of Epsom salt in large (85 gm.) daily doses for an entire year seemed to exert no ill effect. Haag and Palmer,[2] using rats, found that a high Mg diet hindered Ca retention when both Ca and P were low, but that this result was less frequent if either Ca or P were high, and was not apparent when both Ca and P were high. The effects of high Mg on P retention were similar, but not so uniformly depressing when both Ca and P were low, as was the case with Ca retention. High Ca or P, or both, tended to counteract the effects of high Mg. Recently Kramer[3] and co-workers, and also Shipley and Holt[4] have shown that Mg in low concentration can inhibit *in vitro* calcification of rachitic bones. The former workers found that suitable increments of inorganic phosphates overcome the inhibitory action of Mg.

---

* Published with the approval of the Director, as Paper No. 784, Journal Series, Minnesota Agricultural Experiment Station.

† The results here presented are taken from the thesis of Diederick Joh. Schutte for the degree of Master of Science, University of Minnesota, 1928.

[1] Eckles, C. H., Becker, R. B., and Palmer, L. S., *Minnesota Agricultural Experiment Station Bulletin* No. 229, 1926.

[2] Haag, J. R., and Palmer, L. S., *J. Biol. Chem.*, 1928, lxxvi, 367.

[3] Kramer, B., Shelling, B. A., and Orent, E. R., *Johns Hopkins Hosp. Bull.*, 1927, xli, 426.

[4] Shipley, P. G., and Holt, L. E., *Johns Hopkins Hosp. Bull.*, 1927, xli, 437.

It was evident that it had not yet been determined whether $MgSO_4$ is a factor in cattle nutrition under the natural conditions of P deficiency prevailing in Minnesota. Further experimental evidence also seemed advisable with cattle relative to the apparent counteracting effect of phosphates when fed with Mg salts.

The animals used comprised 2 mature cows, E-92 and E-74, which became dry shortly before their first balance period on the basal ration, and 2 heifers, Nos. 392 and 387. E-92 and 392 served as check animals, but the latter, which was pregnant, aborted after the first balance period, and E-92 was used as check animal for No. 387. The average inorganic P content per 100 cc. of blood plasma[5] of the 4 animals at the beginning of the experiment was as follows: E-92, 2.67 mg.; E-74, 3.59 mg.; No. 387, 4.75 mg.; No. 392, 4.05 mg. E-74 and No. 387 were selected for Mg feeding because their blood showed a higher inorganic phosphate content than the corresponding animal of the same age, indicating a superior state of P nutrition. It seemed desirable to give the Mg-fed animals this advantage rather than the check animals. The ration used for all trials was designed to furnish organic nutrients 10 to 20% in excess of the Morrison[6] standard for maintenance, with sufficient P and Ca to maintain an approximate metabolic balance. The ration consisted of prairie hay, grown in the phosphorus-deficient region of Minnesota, ground oats, gluten meal, and cane molasses in varying proportions to meet the individual requirements of the animals. The daily amount of Epsom salt fed amounted to 155-165 gm. This was based upon an average of 0.5% SO_4 content of the well water in the affected regions, and upon an average daily water consumption of 45 lbs. per animal during the winter months. The Mg salt was fed mixed with the molasses, which was poured over the hay.

The feces and urine were collected together. Two adjoining metabolism stalls made possible the conduct of the balance experiments in pairs, in each case an experimental and the check animal. The construction of the stalls was patterned after similar equipment designed by members of the staff of the Pennsylvania Institute of Animal Nutrition.[7] The animals were confined in these stalls for several days before the collection of excreta began. The excreta were weighed and sampled daily, and samples stored in sealed jars

---

[5] Determined by the method of Briggs, *J. Biol. Chem.*, 1924, lix, 255, on 3 consecutive days, using jugular venous blood.

[6] Henry, W. A., and Morrison, F. B., "Feeds and Feeding," p. 746. 18th Edition, Unabridged. The Henry-Morrison Company, Madison, Wis., 1923.

[7] Fries, J. A., Braman, W. W., and Kriss, M., *J. Dairy Sci.*, 1924, vii, 12.

TABLE I.

Ten-day Ca, P and Mg balance of cows during $MgSO_4$ feeding.

Exp.	Period	Animal	Ration	Supplement	Calcium.			Phosphorus.			Magnesium		
					Intake	Outgo	Balance	Intake	Outgo	Balance	Intake	Outgo	Balance
					gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
I	1	E-92	Basal	None	264.2	251.4	+12.8	108.0	75.4	+36.6	154.2	81.7	+72.5
I	1	E-74	Basal	None	281.4	296.2	—14.8	111.9	92.4	+19.5	166.1	92.4	+73.7
I	2	E-92	Basal	None	198.7	186.0	+12.7	54.9	60.0	—5.1	133.8	70.0	+63.8
I	2	E-74	Basal	165 gm. Epsom Salt	171.1	265.4	—94.3	47.2	56.3	—9.1	264.1	213.1	+51.0
II	1	392	Basal	None	309.8	277.1	+32.8	57.9	71.7	—13.8	138.7	70.1	+68.6
II	1	387	Basal	None	309.0	233.3	+75.7	57.1	73.8	—16.7	137.0	53.7	+83.3
II	2	E-92	Basal	None	201.6	180.3	+21.3	47.6	46.4	+1.2	114.5	78.5	+36.0
II	2	387	Basal	156 gm. Epsom Salt	243.9	339.5	—95.6	46.0	71.4	—25.4	298.8	220.6	+78.2
II	3	387	Basal	156 gm. Epsom Salt	253.4	312.6	—59.2	53.5	70.4	—16.9	293.9	219.4	+74.5
II	4	387	Basal	156 gm. Epsom Salt +100 gm. NaH_2PO_4, H_2O	254.1	257.7	— 3.6	283.4	181.3	+102.1	296.8	177.5	+119.3

with chloroform as preservative. The daily aliquots were remixed and sampled at the end of the period.

Each portion of the feed for the entire balance trial was thoroughly mixed and sampled for analysis. Refused portions were also weighed and analyzed. The animals were fed 4 times daily and watered twice daily at regular intervals. Common salt was allowed in liberal amounts. No exercise was permitted during the balance trial but regular brushing was practiced to increase the comfort of the animals. The animals were tested for pica frequently by offering them bleached bones. Inorganic P and Ca of the blood plasma were determined on 3-day composites taken just before and just after each balance trial. Ca was estimated by a modification of the methods of Kramer and Howland,[8] and Kramer and Tisdall[9]; Mg by the method of Howland and Kramer.[10]

Experiment 1 consisted of 2 balance periods with each of the 2 mature cows, E-92 and E-74. Both animals had been on the basal ration for 30 days prior to the first balance period. E-74 received daily 165 gm. of Epsom salt for 35 days before and during Period 2. The results are shown in Table I.

Experiment 2 consisted of 4 balance periods with the experimental heifer No. 387, one balance period with the control heifer No. 392, and one period with the cow E-92. The first balance period was preceded by a 30-day feeding, the second by 42 days on the diets to be tested, the third by 21 days of preliminary feeding, and the fourth balance period immediately followed Period 3. The first period was a basal ration test for both animals. Period 2 was a $MgSO_4$ period for heifer No. 387, preceded by 42 days of Epsom salt feeding, 156 gm. being fed daily. Period 3 was an identical trial run after a further interval of 21 days during which the Epsom salt was fed. In the fourth period, 100 gm. of NaH_2PO_4, H_2O were fed daily, mixed with the grain in addition to the 156 gm. of Epsom salt. The control animal was omitted for Periods 3 and 4 in the experiment because cow E-92, which served as control, had already served in sufficient trials to establish her uniformity of metabolism. The results are shown in Table I.

The variations in the inorganic P and Ca of the blood plasma of the animals are shown in Table II. The data represent the average of the analyses made before and after each balance trial.

The data show conclusively that intakes of $MgSO_4$, comparable to that consumed by cattle in regions where they are forced to drink

---

[8] Kramer, B., and Howland, J., *J. Biol. Chem.*, 1926, lxviii, 74.

[9] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, xlvii, 475.

[10] Howland, J., and Kramer, B., *Am. J. Dis. Child.*, 1921, xxii, 105.

TABLE II.
The effect of Mg on blood Ca and P, in relation to the calcium and phosphorus balances.

Animal	Period	Ration	10-day balance		Inorganic Ca per 100 cc. plasma	Inorganic P per 100 cc. plasma
			P	Ca		
			gm.	gm.	mg.	mg.
E-92	1	Basal	+36.6	+12.8	11.50	2.67
E-92	2	Basal	—5.1	+12.7	12.47	2.24
392	1	Basal	—13.8	+32.8	12.20	4.05
E-74	1	Basal	+19.5	—14.8	11.24	3.59
E-74	2	Basal+165 gm $MgSO_4$, 7 H_2O	—9.1	—93.4	11.66	2.35
387	1	Basal	—16.7	+75.7	12.36	4.75
387	2	Basal+156 gm. $MgSO_4$, 7 H_2O	—25.4	—95.6	10.70	4.35
387	3	Basal+156 gm. $MgSO_4$, 7 H_2O	—16.9	—59.2	10.70	4.66
387	4	Basal+156 gm. $MgSO_4$, 7 H_2O+ 100 gm. NaH_2PO_4, H_2O	+102.1	—3.6	10.43	6.67

water containing this salt, may cause serious and continuous losses of Ca from the body tissues when the P content of the ration is low. The effect of Mg feeding on P balance is not striking, and in these experiments was evident as a detrimental factor only in the younger animal. The ability of the blood system to maintain a nearly constant level of inorganic Ca in the face of severe negative Ca balances is striking. In the case of the older animal receiving $MgSO_4$, the negative Ca balance was accompanied by a decrease in inorganic blood P. Conclusive proof is offered in the data that inorganic P supplements completely overcome the detrimental effect of Mg feeding, both with respect to the effects on Ca and P balance and on blood P. / The large relative storage of Mg in the body of cattle, shown in the data, raises very interesting questions regarding the site of storage of this element.

Some evidence which appears significant was secured with respect to the relation of Mg feeding to the incidence of pica. E-74 and No. 387 began showing evidence of pica soon after receiving Epsom salt in their ration, and the condition became progressively more pronounced as the Mg feeding continued. By the end of the third balance period, No. 387 had developed a severe pica. This decreased greatly within 5 days after the addition of phosphate to her ration and within 10 days she was entirely normal.

4136

Exophthalmia in Trout-Fry.

CHRIS. HAMRE AND M. STARR NICHOLS.
(Introduced by Frederick L. Hisaw.)

From the Department of Zoology and State Laboratory of Hygiene, University of Wisconsin.

H. B. Ward and J. Mueller[1] reported exophthalmia in the trout-fry. Their examination showed the presence of larvae of an unidentified trematode in the muscles of the eyes, at the base of the fins and in some cases in the optic nerves of the protruding eyes. Exophthalmia is prevalent amongst the fish at the State Fish Hatchery at Madison, Wisconsin. Those affected are found to be free from parasites and only those that have been injured are infected with fungus. The condition does not appear until the yolk sac has been absorbed and the fry begin to eat. Large numbers die at this stage of development, and a high percentage of those that die show exophthalmia. The number that die, and the number showing exophthalmia as well, decreases as they grow and summer approaches, only to increase again the following winter and spring when they are a year old. Histological and experimental study show the exophthalmia to be the same in the adult trout and in the fry.

Numerous papers have appeared in the literature describing thyroid diseases of the trout and other fishes, both wild and domesticated. Bonnet[2] first described a tumor of the region of the gill junction, and Scott[3] first described such a tumor as a carcinoma of the thyroid. M. Plehn,[4] L. Pick,[5] Jaboulay,[6] Gaylord and Marsh,[7] Marine and Lenhart,[8] Müller[9] and Johnstone[10] have described similar conditions. In all cases reported, hyperplasia of the thyroid has been indicated externally by a tumor or by areas of hyperaemia. The fry examined by the writers showed no external indication of enlargement of the thyroid.

---

[1] Ward, Henry B., and Mueller, Justus F., *Arch. Schiffs-u. Tropen-Hyg.*, 1926, xxx, 602. (*Biol. Abstr.*, 1927, vii and viii, 1126.)

[2] Bonnet, R., *Bayerische Eisch. Ztg.*, Munchen, 1883, vi, 79.

[3] Scott, *Tr. and Proc. N. Zealand Inst.*, 1891, xxiv, 201.

[4] Plehn, M., *Allg. Fisch. Ztg.*, 1902, vii, 117.

[5] Pick, L., *Berl. Klin. Woch.*, 1905, xlvi-xlix, 1435.

[6] Jaboulay, *J. Med. et Chir. prat.*, 1908, lxxix, 235.

[7] Gaylord, H. R., and Marsh, Millard C., *Bull. U. S. Bur. Fisheries*, 1912, xxxii, 367.

[8] Marine, David, and Lenhart, C. H., *J. Exp. Med.*, 1910, xii, 311.

[9] Müller, F. W., *Virchow's Arch. Path. Anat. u. Physiol.*, 1926, cclx, 405.

[10] Johnstone, J., *Proc. and Tr. Liverpool Biol. Soc.*, 1926, xl, 59.

Exophthalmia was either unilateral of bilateral, the extent of protrusion of the eyes varying from an indication to complete protrusion. When exophthalmia is unilateral, the fry show the phototropic reactions described by Main,[11] in that they tilt along their long axis with the injured eye upward and turn toward the injured eye. If the eyes are equally affected, the tilting reaction is compensated, they swim in a normal manner and turn toward either side with equal frequency. In extreme cases of exophthalmia they are also emaciated and muscularly weak and may be found in 3 stages of the diseased condition: first, indications of exophthalmia, muscular weakness and emaciation; second, more pronounced exophthalmia, and more pronounced muscular weakness in that they remain motionless on the bottom and swim but a short distance when disturbed; third, loss of ability to maintain equilibrium in that they come to rest on their side and respond to stimuli with slow and weak lashings of the tail. They may live in the last condition for some time, the only indications of life being the respiratory movements.

Microscopic sections of the head region show a hyperplastic condition of the thyroid, the alveoli being much enlarged and plicated. The epithelium is definitely columnar and the alveoli lack stainable colloid material. The fry with extreme exophthalmia show a much greater degree of thyroid hyperplasia than those with only an indication of exophthalmus, which indicates a close relation between the 2 conditions.

In order to determine the effect of iodine on the young trout-fry, the fry at the hatchery were divided into 2 groups, one numbering 25,000 and the other 7,000. The larger group received sodium iodide in the water in the proportion of 100 parts to a billion parts of water for 10 days and then 30 parts per billion for the following 8 weeks. The iodine treatment was begun about the time that they began to feed, because, as stated above, the exophthalmic condition does not appear before they have reached this stage of development. The second and smaller group received no iodine in the water but were kept in pools fed by the same spring stratum.

Exophthalmia did not appear in the iodine treated group. The treated fry grew more rapidly than the control fry and at the age of 6 months were found to be 4 times as large as the non-treated fry of the same age. Only occasional fry died, whereas in the 20 years previously practically all the fry died which were hatched at this hatchery unless they were moved to other waters. The thyroid from the fry of the treated group was found to be small, and limited to the region immediately about the ventral aorta. The alveoli were small and distended with stainable colloid material, and the epithelium was of a very low cubical type.

---

[11] Main, R. J., *Z. f. vergl. Physiol.*, 1928, vii, 611.

The alveoli of the fry not receiving iodine and seemingly normal were slightly larger than the alveoli of the fry having received iodine. Likewise, the alveoli were found to be only incompletely filled with stainable colloid material, the walls smooth and free from plications and the epithelium of a cubical type. Gaylord and Marsh describe the thyroids of the trout from Michigan and Wisconsin as being slightly hyperplastic. The fry not receiving iodine, and that died, showed the same microscopic conditions as the exophthalmic fry, indicating that the hyperplastic thyroid was more common than the normal thyroid in the control group.

From the present study it is concluded that iodine prevents the appearance of exophthalmia in trout-fry. The minimum iodine required to prevent the appearance of exophthalmia has not been determined. The iodine as total iodine from the water from the spring stratum was found to be 8.9 parts per billion parts of water. Since very few died that received iodine, it is assumed that the fry not receiving iodine, and that died without exhibiting exophthalmia, died from the same cause for they have been found to lack only the one characteristic. Iodine causes a decrease and a disappearance of exophthalmus, involution of the thyroid gland, the appearance of colloid material in the alveoli as well as an increase in physical vigor. Since the characteristics of the disease do not recur as long as they receive iodine, indications are that it is due to a lack of iodine.

We wish to acknowledge our appreciation for the kind and helpful cooperation of Mr. B. O. Webster, Director of Fisheries, Conservation Commission, Madison, and of Mr. Frank Mead, Superintendent of the Hatchery, who cared for the fry.

4137

Effect of Foreign Protein and of Insulin Administered by Mouth After Ox-Gall.

M. F. GUYER AND P. E. CLAUS.

From the Department of Zoology, University of Wisconsin.

While in the midst of a series of agglutination and precipitin tests on rabbits and guinea pigs the writers came across several news items announcing the success of Professor A. Besredka and his assistant, Dr. Harry Plotz, in securing immunization against typhoid, dysentery and cholera through administering the appropriate vaccine by mouth after a preliminary dose of ox-bile. It seemed worth while to determine whether or not precipitin reactions could be induced by similar treatment and to test the possibility of thus administering insulin. The following experiments were made.

Precipitins. Since experiments were already in progress with antibodies developed against *Beta crystallin* prepared from the lens of the calf, an extract of this protein was used in these tests. In every case, before treating the animals, samples of their blood were taken and a lens precipitin test was made with the serum, using a 1 to 40 dilution of *Beta crystallin* as antigen. The tests were invariably negative. The rabbits were next kept without food for 24 hours, then inspissated ox-gall diluted with 10 volumes of water was administered by means of a stomach tube. Following this, *Beta crystallin* was similarly administered. This procedure was repeated every third or fourth day until 3 such "feedings" were given. Ten days after the last treatment samples of blood were taken and precipitin tests made on them.

Eleven rabbits were used. Of the 9 to which both ox-gall and protein were administered, one died before the final test was made. The serum of the 8 remaining rabbits when tested with a 1 to 40 solution of *Beta crystallin* gave positive titers varying in different animals in dilutions of from 1/320 to 1/5120. The 2 controls, one given protein without the gall, the other gall without the protein, remained negative. In these absorption tests the amount of protein used was about double that ordinarily employed in developing precipitin reactions by intravenous injections.

Insulin by Mouth. When insulin is injected into the blood stream of a normal rabbit it produces a characteristic shock in the animal and the blood sugar falls markedly inside of 2 hours. Using the foregoing method, ox-gall and insulin were administered to 3 rabbits. The blood-sugar of the animals was determined before being fed, then again 2 to 8 hours after, but no perceptible reduction was found. Neither did they display insulin shock. When 6 guinea pigs were similarly treated, however, 5 of them died within 24 hours after treatment, while one, although given ox-gall and insulin 5 times, showed no ill effects. Those which died began to tremble soon after feeding, their hair became ruffled and they refused to eat. The controls, one given gall alone, one insulin alone, remained wholly normal.

Conclusions. Administration of a lens protein *(Beta crystallin)* by mouth following similar administration of ox-gall resulted, in rabbits, in the production of lens precipitins. Insulin similarly administered yielded negative results in 3 rabbits but gave positive shock effects in 5 out of 6 guinea pigs.

4138

A Method for Determination of Lipin Phosphorus.

FRANCES KRASNOW AND A. S. ROSEN.

From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons.

Interest in the *lecithin* content of blood in syphilis necessitated an investigation of the various procedures for the estimation of phosphorus[1-8] or more specifically, lipin phosphorus.[2-6, 9] It was found that "test tube" oxidations by H_2SO_4 and HNO_3 had to be discarded. The danger of bumping with the small amount of reagents used became a source of great concern for micro-determinations.[2-5, 7, 8, 11] This led to the use of H_2SO_4 and H_2O_2 (suggestions of Baumann[11] and Briggs[3]). Digestions were greatly facilitated, but certain difficulties were encountered. The figures obtained were not constant and often too high, the variations depending on the amount of H_2O_2 employed.[6]

After much manipulation, a satisfactory technique was evolved, which may be detailed as follows:

Blood (0.5 cc.) is pipetted into 10.0 cc. of an alcohol-ether mixture (3:1) contained in a 50 cc. volumetric flask, preferably fitted

---

[1] Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920, xliv, 55.

[2] Bloor, W. R., *J. Biol. Chem.*, 1915, xxii, 133; 1916, xxiv, 447; 1915, xxiii, 317; 1918, xxxvi, 33.

[3] Briggs, A. B., *J. Biol. Chem.*, 1924, lix, 255.

[4] Greenwald, I., *J. Biol. Chem.*, 1915, xxi, 29.

[5] Grigaut, M. A., *J. de Pharmacie et de Chimie*, 1925, 8th series, i, 97.

[6] Krasnow, F., and Rosen, A. S., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 352.

[7] Pouget, I., et Chouchak, D., *Bull. Soc. chimique de Paris*, 1909, series 4, v, 104.

[8] Tisdall, F. F., *J. Biol. Chem.*, 1922, l, 329.

[9] Randels, F. S., and Knudson, A., *J. Biol. Chem.*, 1922, liii, 53.

[10] Whitehorn, J. C., *J. Biol Chem.*, 1924, lxii, 133.

[11] Baumann, E. J., *J. Biol. Chem.*, 1924, lix, 667.

with a glass stopper, and brought to boiling by immersion in a water-bath. Digestion is continued for about 3 min. After cooling, alcohol-ether is added to the 50 cc. mark and the whole shaken vigorously. The filtrate (25 cc.) is transferred quantitatively to a casserol of 150 cc. capacity and evaporated to dryness over a water-bath. Then 10 cc. of HNO_3 (sp. gr. 1.42) is added and the dish rotated in such a way that the acid loosens the dried extract from the sides. 0.1 cc. H_2SO_4 (sp. gr. 1.84), 5 cc. $KClO_3$ (saturated solution) and 4 cc. of a mixture of saturated KNO_3 and a saturated solution of $NaNO_3$ (1:1) are introduced. The casserol is placed in the oven at 85-95° C. and digestion continued until the mixture becomes white. A safe interval is about 20 hours. A longer period does not matter. The white crystalline residue is dissolved in water by heating over a small free flame and transferred to a centrifuge tube (15 cc.). Including at least 2 washings, the total volume should not exceed 10 cc.

Following the Tisdall method,[8] the phosphate is precipitated with 1 cc. of the strychnine-molybdate reagent and stirred 3 times during 15 min. After being centrifuged for 3 minutes at high speed, the supernatant fluid is decanted and the precipitate washed twice with water, each time centrifuging one minute. 0.5 cc. NaOH (1%) is added and stirred until solution is complete. This is diluted with water to about 2.5 cc. and transferred to a 25 cc. volumetric flask fitted with a glass stopper. The centrifuge tube is washed twice with water, using 2.5 cc. each time and the washings added. 5 cc. K_4FeCN_6 solution (20%) and 2.5 cc. HCl (sp. gr. 1.18) are introduced. The mixture is allowed to stand for 10 to 12 minutes and made up to volume with water. Readings are made immediately against a phosphate standard, prepared as directed by Tisdall. The formula for calculating the lipin phosphorus, P, in 100 cc. of whole blood is: $P = 20/R \times 5$ where 20 is the reading of the standard and R the reading of the unknown. To obtain the corresponding *lecithin* content, the value of P is multiplied by 25.

Tests have shown that the blood may be kept over night in the ice-chest and the procedure outlined above may be conveniently interrupted at any point except after the addition of the concentrated HCl. Continued study is indicating that only slight modifications will be necessary to enable us to apply this technique to the determination of total phosphorus in blood and other biological fluids.

4139

On the Vitality of Dental Enamel.

WILLIAM J. GIES.

From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons.

Various common pathological changes in the dental enamel of fully erupted human teeth, which destroy its protective function, are ascribed by some investigators to intrinsic nutritional alterations, rather than to extrinsic influences. The proof that enamel contains protein[1, 2] has induced several investigators to regard its presence as indicative of nutritional capacity, despite the fact that, although permeable to water and various substances, enamel completely lacks power of self-repair after injury and has no cellular nuclei. That the contained protein (probably keratin) is not associated with intrinsic nutritional changes in the enamel of erupted teeth is indicated by the writer's further observation that conventional tests for proteolytic and amylolytic enzymes, applied to extracts of pulverized normal enamel, were completely negative. These tests, to be extended, will include studies of abnormal enamel.

4140

Occurrence of Cystine in Sweat of Cystinurics.

HOWARD B. LEWIS.

From the Laboratory of Physiological Chemistry, Medical School, University of Michigan.

In 1871, Dewar and Gamgee[1] stated that, in some cases, cystine was present in the sweat of cystinurics, apparently basing their conclusions largely upon the observation that, in cases where cystine was present in the urine, silver coins carried in the pocket were observed rapidly to become blackened. Garrod,[2] however, was unable to obtain evidence of the presence of cystine in the sweat in the one case studied by him.

---

[1] Bödecker and Gies, PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 175.

[2] Gies, *J. Dental Res.*, 1926, vi, 143.

[1] Dewar, J., and Gamgee, A., *J. Anat. Physiol.*, 1871, v, 142.

[2] Garrod, A. E., "Inborn Errors of Metabolism," London, 1923, 2nd edition, p. 103.

We have had under observation a cystinuric whose daily cystine excretion (determined by the colorimetric method of Folin and Looney) varied from 1.0 to 1.8 gm., whose urine frequently contained large amounts of cystine crystals, and who occasionally passed cystine calculi. From this patient we collected by the use of pilocarpine and heat, 100 cc. of sweat in 50 minutes.* No cystine could be detected by the colorimetric method of Folin and Looney. 35 cc. of sweat were deproteinized by the use of heat and alumina cream and the filtrate was evaporated to dryness. The residue was extracted with dilute ammonium hydroxide and the extract treated with acetic acid. No cystine crystals were observed microscopically. Wollaston's test (formation of cystine hydrochloride crystals) was also negative.

4141

Factors in the Control of Ascites.

GEORGE M. CURTIS AND C. B. HUGGINS.*

From the Department of Surgery, University of Chicago.

Ascites associated with portal cirrhosis of the liver is ordinarily controlled in one of 3 ways: by parcentesis, by variations of the Talma-Morison operation, or by the diuretics. All 3 procedures were employed in this case-study—a man of 58 with a long alcoholic history; a slight jaundice, malaise and some edema of the legs during the past 7 months; and an ascites of 2 months' duration. Paracentesis was made 5 times in all in the 8 weeks previous to operation, from 9 to 14 liters of fluid being removed each time. Analyses were made of the fluid. Omentopexy was accomplished 10 weeks after the onset of the ascites. A large fan of the omentum was brought out through a mid-line incision in the upper abdomen, spread out and sewn in place in a prepared properitoneal bed. The peritoneum was closed about the omental stalk with wide-apart mattress sutures. Convalescence was uneventful. The diagnosis of portal cirrhosis was confirmed at operation.

The reaccumulation of fluid in the peritoneal cavity subsequent to various attempts to establish additional collaterals is a frequent ex-

---

* We are indebted to Dr. Robert Barney of the Department of Dermatology and Syphilology for the collection of the sample of sweat.

* This work has been conducted in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

perience. It occurred even in Morison's first case.[1] Thus paracentesis postoperatively may often be necessary. Here, however, we substituted the action of specific diuretics, especially novasurol (merbaphen) as investigated by Keith and Whelan,[2] in an attempt to solve this post-operative problem. A particular study of the action of the diuretics was made during a 17 day period commencing 23 days after the omentopexy.

The usual amount of phenolsulphonephthalein (6 mgm.) injected intraperitoneally at the time of a preoperative paracentesis was slowly excreted by the kidney, appearing in the urine during the second hour; but 13% being excreted in 4 hours. This was in accord with the small quantity of urine excreted in spite of a normal water intake.

During the 17 day period each specimen of urine was measured as voided and its chloride concentration determined. The rate of water and chloride excretion was calculated. During the entire period the patient was maintained on a low salt diet and limited fluid intake. Upon institution of this regimen there was a slight fall in the rate of chloride excretion, the water excretion remaining about the same. The preliminary injection of 0.5 cc. of novasurol resulted in a very slight increase in both rates. 1.2 cc. of novasurol the following day caused a definite but comparatively small increase in both rates. During this preliminary period the sodium chloride concentration of the urine varied between 0.75 and 1.2%.

Ammonium chloride, 2 gm. 4 times daily, over a period of 4 days resulted in a gradual rise of the chloride excretion and no marked change in the rate of water excretion. The sodium chloride concentration of the urine rose during this period from 0.65 to 1.63%. Novasurol, 2.0 cc., administered intravenously at the end of this 4 day period resulted in a striking increase of both water and chloride excretion.

Theophylline dethylenediamine (Euphyllin) administered intramuscularly or intravenously in 2.0 cc. doses had but little effect upon the rate of water and salt excretion, even when given during ammonium chloride administration. Increase of the dose to 3.0 cc. was likewise without marked action. Paracentesis in itself caused no increase in the rates; combined with Euphyllin there was a slight increase. Both the preoperative ascitic fluid and that following the diuretic study had a higher content of albuminous substance than that obtained during the period of ammonium chloride and diuretic administration. During the 17 day period the weight was reduced from 100.2 to 86.0 kg.

---

[1] **Drummond and Morison, *Brit. Med. J.*, 1896, ii, 728.**

[2] **Keith and Whelan, *J. Clin. Invest.*, 1926, iii, 149.**

Upon cessation of the administration of the diuretics the ascites returned. It is questionable how great a collateral circulation was established by operation. However, the postoperative use of novasurol and ammonium chloride was found valuable as a substitute for the customary paracentesis.

4142

Vitamin Requirements of Nursing Young (Mus norvegicus albinus). V. Hypoglycemia in Nursing Young Suffering from Vitamin B Deficiency.*

BARNETT SURE AND MARGARET ELIZABETH SMITH.

From the Departments of Agricultural Chemistry and Home Economics, University of Arkansas, Fayetteville.

Recently Sure, Kik and Walker[1] have reported anhydremia associated with disturbance in hematopoietic function in nursing young of the albino rat suffering from vitamin B† deficiency. For the past six months we have been engaged in a study of the effect of a deficiency of the vitamin B complex† of the lactating mother on the total blood sugar content of the nursing young. Although the results of our work are not yet complete, we think it of interest at this time to report on one pathological litter compared with a control litter. The mother of the control litter received our vitamin B deficient ration No. 1009[2] and in addition a separate administration daily of 1000 mg. of Northwestern dehydrated yeast from the time the young were 5 days old. The maternal diet of the pathological litter consisted of the same ration No. 1009 but the mother received only 300 mgm. daily of the same yeast from the day the litter was born until the 21st day of lactation. At this time the first maintenance point of the litter was encountered. After several days slight growth followed, the daily dosage of yeast to the mother was

---

* Research Paper No. 67, Journal Series, University of Arkansas.

[1] Sure, B., Kik, M. C., and Walker, D. J., *Proc. Am. Soc. Biol. Chem.*, 1928, lxxviii, 18.

† The term "vitamin B" is used in this paper to signify the combination of the labile-antineuritic and the stable factors associated with the vitamin B complex, both of which are necessary for growth.

[2] Sure, B., *J. Biol. Chem.*, 1928, lxxvi, 676.

gradually reduced to 100 mg. In order to establish prolonged maintenance, the mother was later allowed, instead of the dehydrated yeast, a daily dosage of 20 mg. of one of our less potent yeast concentrates. The pathological condition of the young was then produced on a prolonged maintenance level.

The total blood sugar of the control group showed a definite increase during the nursing period, rising from approximately 100 mg. on the 15th day of lactation to approximately 130 mg. at weaning. In the pathological group there was a decrease in blood sugar, during the prolonged maintenance period, as follows: 19, 40, 24, 20, 22 and 24% respectively of total blood sugar for each animal.

4143

On the Pathological Relations Between Cancer and Tuberculosis.

RAYMOND PEARL.

From the Institute for Biological Research of the Johns Hopkins University, and the Statistical Department of the Johns Hopkins Hospital.

For some time past we have been engaged in a biometrical and experimental investigation of the relation between cancer and tuberculosis.* In the biometrical study there was first set up, by a critically adequate procedure, a case-for-case control series of autopsied patients, each one of whom was of the same sex, age and color (race) as one of the 816 persons autopsied in the Johns Hopkins Hospital and found to have carcinoma, sarcoma, or some other form of malignant tumor. The individuals in the control series did not have cancer or any other malignant tumor. With these malignant and non-malignant series of cases it was then shown that:

1. Active tuberculous lesions were found at autopsy in only 6.6% of 816 persons having malignant growths. On the other hand such tuberculous lesions were found in 16.3% of 816 persons without malignant tumors, but of the same race, sex, and age as the former group. Active tuberculous lesions therefore occurred over twice as frequently in the controls as they did in the malignant group in the present material taken as a whole.
2. Active tuberculous lesions occur 2.2 times more frequently among the controls of the carcinomatous than among those having carcinoma; whereas active tuberculous lesions occur 3.3 times more

---

* The biometrical results will be published in full in the *Am. J. of Hygiene.*

frequently among the controls of the sarcomatous than among those having sarcoma.

3. Healed tuberculous lesions (apical scars, calcified tubercles in glands, etc.) occur with equal frequency in the malignancy and control groups, whether they are taken as wholes, or when carcinoma and sarcoma (and other) are separately treated.

4. In each decade of age, and in each sex and race division of the material, the percentage of persons showing active tuberculous lesions at autopsy is markedly higher in the control group than in the malignancy group.

5. Looking at the matter from the reverse aspect, it was found that, in this autopsy experience, persons who had tuberculous lesions of any degree of activity whatever, showed a relative incidence of cancer and other malignant tumors, only a little more than half as great as that exhibited either by persons with no tuberculosis discoverable at autopsy, or by those who have only old, healed tuberculous lesions.

6. In each decade of age, over the whole life span, cancer or other malignant tumors occur less frequently in those with active tuberculosis than in either the non-tuberculous or those with old healed lesions.

7. In 886 persons of both sexes and races compared, *who had a great deal of florid, active tuberculosis* (acute, ulcerative tuberculosis of the lungs or intestines, tuberculous meningitis, caries of the vertebrae, etc.) there were but 11 cases of malignant tumors, or 1.2% of the total number. On the other hand, in 886 persons with no recorded lesions of tuberculosis at autopsy, having the same age, sex, and racial distribution, case for case, as the very active tuberculous, there were 82 cases of malignant tumors, or 9.3%. Among the females, both white and colored, there was not a single case of malignant growth in those with much active tuberculosis. This general result is confined in each separate age, sex, and color group, save for a few cases where the numbers are too small to give any reliable indications whatever.

8. There is no significant difference in this material between groups of persons with cancer or other malignant tumors, and groups of persons without any malignant neoplasms, in respect of the incidence of either pleural adhesions or emphysema, all of the persons in both groups having no lesions of tuberculosis.

9. Five general classes of facts derived from official mortality statistics from the general population are in agreement, in principle, with the conclusions reached above from autopsy material. The

same is true of the results of a study of the correlation of age specific death rates in connection with this problem.

10. In general, it must be concluded that there is a definite and marked incompatibility or antagonism between the 2 diseases, cancer and tuberculosis, of such sort that both occur together at the same time in florid activity in the same individual only with great rarity.

Some years ago a Scandinavian clinician, Isager,[1] was so impressed with the antagonism between cancer and tuberculosis in his clinical experience that he made the suggestion that the experiment should be tried of using tuberculin to test the progress of cancerous disease. Another obvious suggestion is to try the experiment of treating inoperable cases of cancer with tuberculin. The ideal case for a trial would be one in which, first, a cancer had been removed surgically, and the malignant character of the tumor definitely proven from sections of the surgical specimen; in which, second, there had been at some later date an extensive, inoperable recurrence of the same tumor, confirmed by biopsy. If in such a case the tumor retrogressed or disappeared, following tuberculin treatment alone, the evidence would have real significance.

---

[1] Isager, *Hospitalstid,* 1922, lxv, 481.